OPINION

Exit from dormancy in microbial organisms

Jonathan Dworkin and Ishita M. Shah

Abstract | Bacteria can exist in metabolically inactive states that allow them to survive conditions that are not conducive for growth. Such dormant cells may sense when conditions have improved and re-initiate growth, lest they be outcompeted by their neighbours. Growing bacteria turn over and release large quantities of their cell walls into the environment. Drawing from recent work on the germination of *Bacillus subtilis* spores, we propose that many microorganisms exit dormancy in response to cell wall muropeptides.

The ability of microorganisms to persist in metabolically inactive states enables survival in unfavourable conditions[1] and probably contributes to microbial diversity by facilitating taxonomic richness in nutrient-poor systems[2]. Unfavourable conditions include nutrient deprivation, extremes of temperature and desiccation, or the presence of host antimicrobials such as lysozyme[3]. Several important human pathogens can exist in these states[4] and, at least in some cases, the infectious particles are dormant cells[5]. For example, *Bacillus anthracis* and *Clostridium difficile* can become resistant spores[6], *Mycobacterium tuberculosis* can enter into a low replicative state that may aid survival in human hosts for decades[7,8], and *Chlamydia* spp. are capable of transforming into an inert form, the elementary body[9]. A similar survival strategy is seen in many environmental isolates[10], such as *Pseudomonas fluorescens*[11] and *Vibrio vulnificus*[12], which enter dormant states that improve their long-term survival in the soil and in cold aquatic environments, respectively.

Although stochastic mechanisms could underlie the decision to exit these low- or zero-growth physiological states, dormant cells might also determine when conditions have improved and re-initiate growth. Given that bacteria typically exist in polymicrobial communities, dormant cells should efficiently and accurately assess the changes in environmental conditions to be able to compete with genetically related bacteria or phylogenetically distinct species that are present in these settings. Although eukaryotic microorganisms such as fungi are beyond the scope of this article, they also undergo exit from dormancy (BOX 1). Little is known about the mechanistic basis for this transition, but, given that many important fungal pathogens initiate infection as dormant propagules, this question deserves further investigation.

The presence of nutrients has been considered a key signal that stimulates exit from dormancy. However, bacterial cells can also exit dormancy in response to signals released by other bacteria[13,14]. We have recently found that exit from dormancy in *Bacillus subtilis* spores (germination) occurs in response to peptidoglycan fragments released by growing *B. subtilis* cells[15]. Here, we describe these findings, as well as related observations on the resuscitation of dormant *M. tuberculosis* cells, and propose how peptidoglycan fragments might have a role in the exit from dormant states in phylogenetically diverse bacteria.

What are dormant bacteria?

A range of environmental conditions can cause growing bacteria to sense that their surroundings are incapable of supporting continued growth. These include nutrient starvation or limitation, toxic chemical concentrations and changes in temperature or pressure. Bacterial species respond to these conditions in several ways, some of which involve clear morphological differentiation (for example, spore formation) and others of which are not so morphologically distinct. However, all these responses result in a notable reduction in metabolism, to the point of absolute dormancy in some cases. For the purposes of this article, we define dormancy as levels of metabolic activity that are undetectable under normal laboratory conditions, being mindful that these conditions might not be representative of the organism's actual environment, such as the soil. Below, we describe some of these different responses and what is known about the environments that lead to these transformations, but it should be noted that the mechanistic basis for most of these states is not well understood, so it is not possible to unambiguously relate the different states to each other.

Bacterial spores. *Bacillus* spp. and *Clostridium* spp. respond to nutrient limitation by undergoing a complex process referred to as sporulation. In the laboratory, entry into sporulation is stimulated by a broad nutritional downshift, inhibition of GTP synthesis or growth until exhaustion in a defined rich medium. Although the sensor kinases that are necessary for this stimulation have been identified, their ligands remain unknown and so the physiologically relevant conditions remain mysterious. Sporulation involves tightly regulated transcriptional changes and subsequent dramatic morphological changes that result in the production of a dormant, environmentally resilient spore. The spore can remain dormant for many years and can withstand high temperatures, desiccation, radiation and toxic chemicals. The molecular basis of spore dormancy is not completely understood, but the physical state of water[16] and lipids[17] in the different spore compartments is thought to have a key role. In addition, the DNA exists in a compacted state, which is facilitated by the action of DNA-binding small, acid-soluble spore proteins (SASPs) and provides resistance to ultraviolet (UV) light-induced mutagenesis[18]. The robustness of spores probably facilitates their action as infectious particles, and it was recently

reported that mycobacteria can form spore-like structures[19], although other investigators have failed to replicate these findings[20].

Intracellular human pathogens. The ability of some bacteria to maintain an extended and apparently stable dormant intracellular state is thought to facilitate their pathogenesis. For example, *Chlamydia* spp. are obligate intracellular bacteria that exist as either a metabolically inert elementary body or a replicating reticulate body[21,22]. The electron-dense elementary body contains a condensed nucleoid and is thought to be the form that allows persistent, long-term infection. Elementary body formation *in vivo* is not well understood, although it can be triggered by stimuli such as nutrient limitation in the vacuoles, where the bacteria replicate[23], or by host factors such as cytokines[21]. Another intracellular pathogen, *Coxiella burnetii*, is highly resistant to environmental conditions, mainly because it can form a stable small-cell variant that is much less metabolically active than the large-cell variant[24]. *Coxiella* spp. can cycle between these cell types, but the trigger (or triggers) for these transitions are unknown. Although *C. burnetii* has long been thought to be an obligate intracellular bacterium, the recent identification of conditions permissive for host-free growth[25] will aid the investigation of these transitions and of the enhanced robustness of the small-cell variant. The causative agent of Lyme disease, *Borrelia burgdorferi*, enters a dormant state in host cells called the 'round body', which is induced by environmental conditions that are unfavourable to growth and is thought to facilitate persistence of this bacterium in the host[26]. Finally, the ability of the major human pathogen *M. tuberculosis* to enter dormancy is probably directly relevant to its pathogenesis[27,28], as the bacterium can exist in the human host for >40 years before reactivating[7]. However, an unresolved question is how this clinically defined phenomenon of latency relates to the phenomena of dormancy and culturability[29].

Viable but non-culturable states. The exposure of growing cells to environmental stresses can result in a decline in the cultivability of these cells instead of cell lethality[30]. These 'viable but non-culturable' (VBNC) cells fail to grow under many laboratory conditions but are alive and capable of growth when subject to a subset of conditions such as rich medium or filtrate from growing cells. This was first studied in *Vibrio cholerae*[10], but a phylogenetically diverse range of species, including numerous important pathogens, can enter VBNC states that are thought to enhance survival under harsh conditions[5]. These species include *Salmonella enterica* subsp. *enterica* serovar Typhi, which can survive and remain infectious despite long periods (~9 months) of desiccation[31], *Legionella pneumophila*[32], stationary phase forms of which are stable under nutrient-poor conditions and undergo host-dependent differentiation on infection[33,34], and *Micrococcus luteus* when it is grown under nutrient-limiting conditions[35]. It remains controversial, however, whether the VBNC state is a true physiological state or whether this classification simply reflects our limited understanding of the nutritional requirements for bacterial growth[36].

Persisters. For many bacterial species, a fraction of their population has increased tolerance to multiple antibiotics, and these bacteria are called persisters. This tolerance results from the absence of growth — so that processes such as cell wall synthesis or translation, which are typical antibiotic targets, are largely inactive — and not from the acquisition of specific mutations that render the bacterium insensitive to these antibiotics. The presence of persisters in a biofilm probably accounts for the observed antimicrobial tolerance of these communities and could therefore be important in the aetiology of many recalcitrant infectious diseases[37]. Examples include slow-growing small-colony variants of *Staphylococcus aureus* and of several phylogenetically diverse species[38].

Compared with VBNC cells, persisters constitute only a small fraction of the population, and although this state does not seem to be a response to environmental stimuli, little is known about how persisters are formed. In *Escherichia coli*, the kinase HipA phosphorylates the translation factor elongation factor-Tu (EF-Tu) *in vitro*, and *hipA* mutants have increased rates of persister formation[39]. Another mechanism that has been proposed for persister formation is increased expression of chromosomal toxin–antitoxin proteins[37], some of which can inhibit translation. It is unclear, however, whether either of these mechanisms is relevant to multiple species.

Exit from dormancy

As with entry into dormancy, our limited knowledge of the conditions in which bacteria exist, either in hosts or in the environment, precludes the definitive identification of the stimuli for exit from dormancy. Exit can be a stochastic event that is unaffected by any external stimuli, as seems to be the case with persisters[40]. However, it is useful to consider why microbial cells might exit dormancy and how they might detect permissive environmental conditions. Although the presence of nutrients is consistent with growth-supporting conditions, this reflects only one characteristic necessary for growth. For example, in a host, in addition to adequate nutritional sources there might be high concentrations of antimicrobials such as lysozyme, which target processes necessary for growth and make the environment inhospitable for non-dormant cells. Dormant cells therefore need to identify growth-promoting conditions, but the wide range of possible conditions makes such a determination challenging. In the following section, we address the possibility that one signal of such conditions is the release of cell wall muropeptides (FIGS 1,2) by other growing microorganisms.

Box 1 | **Dormancy in eukaryotic microorganisms**

Fungal asexual spores (conidia) saturate the air we breathe to amounts reaching thousands of spores per cubic metre[93]. Conidia are haploid, dormant, desiccated cells that can survive adverse environmental conditions and typically serve as the infectious propagules[94]. Conidial germination is required to initiate both infection and asexual development, and although the morphological transitions during asexual development have been well characterized, the molecular mechanisms that underlie conidial germination have remained largely elusive[95].

Many fungal species can exist in a dormant state, including *Saccharomyces cerevisiae*[96,97], *Schizosaccharomyces pombe*[98], *Aspergillus fumigatus*[99], *Neurospora crassa*[100] and *Cryptococcus neoformans*[101]. It is possible that, like bacterial spores, conidial germination is dependent on cell wall-associated proteins that transmit environmental signals from the external environment to inside the cell. Disrupting these proteins would prevent dormant conidia from sensing their external environment, and they would therefore not receive the necessary 'wake-up' signals required to exit dormancy. The fungal cell wall is composed of β-glucan polymers and, by analogy with peptidoglycan muropeptides, β-glucan released by growing fungal cells[102] could serve as a signal for growth-permissive conditions. Interestingly, bacteria-derived muropeptides strongly promote *Candida albicans* hyphal growth, indicating that these molecules might serve as inter-kingdom signals[103].

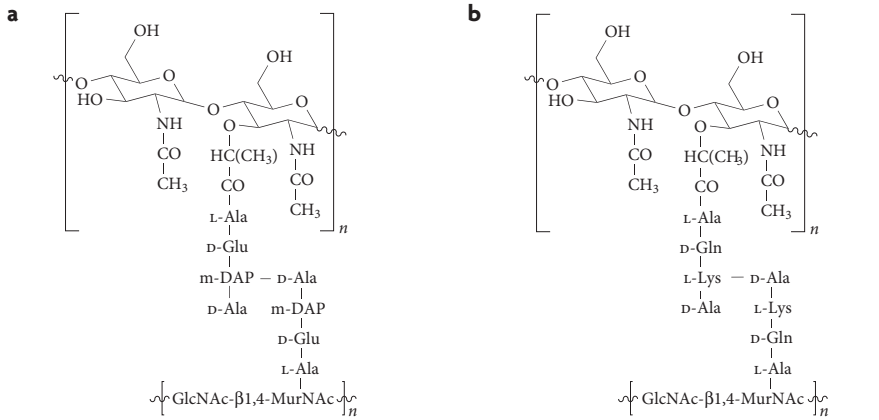

Figure 1 | **Peptidoglycan structure. a** | The polysaccharide backbone of peptidoglycan is linked by stem peptides. Gram-negative bacteria and Gram-positive Bacilli contain meso-diaminopimelic acid (m-DAP) as the third amino acid in the peptide linker. **b** | Most other Gram-positive bacteria (including Gram-positive cocci) contain L-lysine as the third amino acid in the linker. GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid.

Germination of spores triggered by muropeptides. *Bacillus* spp. spores germinate in the laboratory in the presence of amino acids such as L-alanine (alone or in combination with glucose)[41], although the concentrations of amino acids required (typically millimolar) are probably non-physiological. Germination is dependent on proteins — known as germination receptors — that have been proposed to bind these molecules[41]. Germination receptors are absent from clostridia, and spores of *C. difficile* germinate in response to ligands found in host environments, such as bile salts found in the gastrointestinal tract[42,43].

B. subtilis spores also germinate in response to micromolar concentrations of peptidoglycan-derived muropeptides consisting of a disaccharide–tripeptide with a meso-diaminopimelic acid (m-DAP) residue at the third position of the stem peptide[15] (FIG. 1a). Growing bacteria, especially Gram-positive species, release large quantities of peptidoglycan-derived muropeptides into the culture medium[44]. As peptidoglycan is an extremely well-conserved molecule, these muropeptides could serve as an interspecies signal of the presence of conditions that support microbial growth. Consistent with the information provided by this signal, peptidoglycan derived from growing cells is a much more effective inducer of germination than that derived from cells in extended periods (~24 h) of stationary phase[15]. This effect is reminiscent of the immunostimulatory nature of supernatants that contain peptidoglycan fragments from growing bacteria[45] and of muropeptides that are shed during *Shigella* spp. infection[46], and it is also similar to the role of these molecules in the host detection of pathogens[47].

As stochastic events seem to play a part in the exit from dormancy of some species (for example, *E. coli* persisters[40]), one question raised by the stimulation of germination by muropeptides is whether spontaneously germinating spores can also stimulate neighbouring spores to germinate and thereby induce a positive feedback-mediated 'chain reaction' of germination. Although this scenario is appealing from the perspective of efficiency, the lack of correlation with environmental parameters could lead to extensive germination under inappropriate conditions. This dilemma seems to have been solved, however, as peptidoglycan released by germinating spores does not act as a germinant (J.D. and I.M.S., unpublished observations). Although spore peptidoglycan is different from vegetative peptidoglycan[48], the particular modifications (or lack thereof) that inhibit its ability to stimulate germination remain to be identified.

Role of a conserved membrane kinase in detecting muropeptides. The germination response to muropeptides requires a highly conserved, eukaryotic-like membrane serine/threonine kinase, PrkC, the extracellular domain of which contains PASTA repeats that are responsible for peptidoglycan binding. This response is independent of other identified germination receptors that mediate the response to alanine and other amino acids. During germination, PrkC phosphorylates the essential translation factor EF-G[15], as it does in growing cells[49]. EF-G is phosphorylated in a range of bacteria[50], although the functional consequences of this modification are not known. Dormant spores of *Bacillus megaterium*[51] and chlamydial elementary bodies[52] contain large amounts of mRNA and ribosomes, so translation stimulation might be a fundamental mechanism of exit from dormancy. Presumably, this stimulation is specific to mRNAs encoding proteins that are necessary for the earliest and most essential processes underlying exit from dormancy. Consistent with this possibility, dormant spores of the anaerobe *Clostridium novyi* NT are enriched for transcripts that encode proteins with redox activity necessary for growth[53].

EF-G is necessary not only for the translocation of mRNAs and transfer RNAs in the ribosome during translation, but also, in conjunction with ribosome release factor (RRF), for the process by which the 70S bacterial ribosome is split into the 30S and 50S subunits in preparation for a new round of translation[54,55]. In *E. coli*, this process is necessary for the transition from stationary phase to growth, and deletion of RRF causes cells to die during this transition[56]. The bacterial protein Y (pY) family of proteins — for example, *E. coli* ribosome-associated inhibition factor A (RaiA) — bind 70S ribosomes and prevent disassembly of the 70S complex[57]. This has the effect of forming pools of idle 70S ribosomes and therefore reducing the translational capacity of the cell[58]. Recently, EF-G and RRF were shown to work together to remove the chloroplast protein plastid-specific 30S ribosomal protein 1 (PSRP1), the pY functional analogue, from ribosomes in response to light stimulation[59]. Thus, phosphorylation of EF-G could enhance its ability to offset the inhibitory effect of pY proteins on translation and would thereby be a trigger for germination and, in general, for exit from dormancy.

Proteins with substantial homology to PrkC in both the intracellular kinase domain and the extracellular peptidoglycan-binding domains (FIG. 3) are found in most, if not all, Gram-positive species[60], including *S. aureus*[61,62], *Streptococcus pneumoniae*[63] and *M. tuberculosis*[64], in which these proteins are involved in cell wall metabolism and have important but ill-defined roles in bacterial pathogenesis. Substitution of *B. subtilis* PrkC with the homologue from *S. aureus* also allows *B. subtilis* spores to germinate in response to peptidoglycan, suggesting that this kinase also detects muropeptides and phosphorylates EF-G. Interestingly, spores expressing this kinase respond to both m-DAP-containing and L-Lys-containing

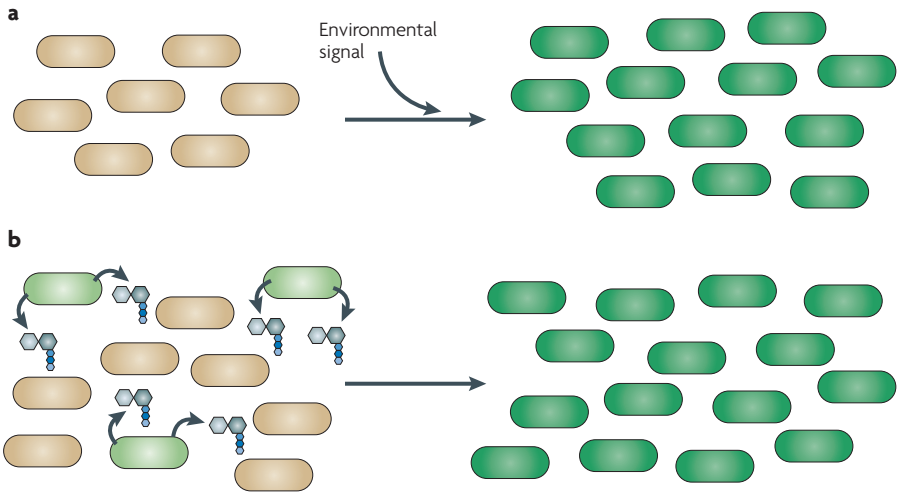

Figure 2 | **Exit from dormancy triggered by growing cells. a** | Dormant cells (beige) may sense some aspect of the environment before exiting dormancy and initiating growth (green cells). **b** | The presence of secreted signalling molecules such as cell wall muropeptides from growing cells (pale green) could serve as an indication that growth-permissive conditions are present and could thereby stimulate exit from dormancy.

muropeptides, suggesting that bacteria expressing this kinase can receive signals from all species that produce peptidoglycan.

Finally, the detection of cell wall fragments in the environment might be a widely used strategy in the microbial world[47]. For example, *Chlamydia* spp. express genes encoding members of the peptidoglycan biosynthesis pathway despite lacking a detectable cell wall[65]. This 'peptidoglycan anomaly' suggests that *Chlamydia* spp. might use peptidoglycan as a signalling molecule and not as a structural component.

Actinobacteria. As mentioned above, *M. luteus* can enter a dormant state under starvation conditions[35]. In a crucial advance, a protein known as resuscitation-promoting factor (Rpf) was found to stimulate the growth of these dormant cells when added extracellularly[66]. *M. luteus* Rpf is a potent secreted factor with the ability to hydrolyse peptidoglycan at picomolar concentrations[66–68], and this enzymatic activity is necessary for the stimulation of exit from dormancy[68]. This ability seems to be well conserved, as *M. luteus* Rpf stimulates the growth of aged cultures of *M. tuberculosis*[69].

Five endogenous Rpfs have been identified in *M. tuberculosis*. These proteins have been shown to be crucial for reactivation from chronic tuberculosis in animal models[27,70], and their muralytic activity has been proposed to be essential for stimulating reactivation[8]. However, there are conflicting *in vivo* and *in vitro* results about whether these Rpfs are functionally redundant[29].

Rpfs interact with proteins involved in cell wall metabolism, including another peptidoglycan hydrolase, RipA[71,72]. It is unclear whether Rpf-mediated peptidoglycan hydrolysis is necessary simply to allow the physical changes in cell morphology that are required for growth, as is the case in *B. subtilis* spore germination[48], or whether these fragments have additional signalling functions. Consistent with this latter possibility, *M. tuberculosis* encodes PknB, a PrkC homologue that also contains peptidoglycan-binding PASTA repeats. It has been suggested that PknB responds to muropeptides that are generated as a result of the muralytic activity of Rpf proteins[29,60] and initiates changes necessary for exit from dormancy. In support of this possibility, an Rpf-like protein from *B. subtilis* (the secreted hydrolase YocH) stimulates the expression of specific genes in growing cells in a manner that is dependent on the presence of PrkC[73]. Several targets of PknB have been reported, including proteins containing forkhead domains[74] and, intriguingly, two proteins involved in peptidoglycan synthesis (penicillin-binding protein A (REF. 75) and GlmU, an *N*-acetylglucosamine-1-phosphate uridyltransferase[76]), suggesting that PknB has a role in regulating cell division[63,77]. As this process is necessary for growth, one plausible mechanism of dormancy exit is that PknB-mediated phosphorylation of these targets in response to exogenous muropeptides leads to stimulation of division and growth.

This mechanism seems to be different from that described above for the phosphorylation of EF-G by PrkC. However, the highly conserved nature of EF-G and the ability of a PrkC homologue, *S. aureus* PknB, to substitute for PrkC in germination[15] suggests that *M. tuberculosis* PknB also phosphorylates EF-G and that these two pathways therefore operate in parallel. The recent report that PrkC phosphorylates key metabolic enzymes in *B. subtilis*[78] suggests that, in this bacterium, pathways other than translation are subject to regulation through phosphorylation. Future work will investigate how (and whether) modifications to EF-G and to biosynthetic enzymes function convergently in regulating cell growth. However, it is clear that pathways sensitive to peptidoglycan have key roles in the exit from dormancy in phylogenetically diverse bacteria.

Role of other intercellular signals

Bacteria respond to signals generated by other bacteria[14,79,80], and the information transmitted is often an assessment of population density[81], a phenomenon referred to as quorum sensing. These signals probably also reflect other aspects of the environment, as population density directly reflects past growth[82]. Because the signals that mediate quorum sensing are affected by environmental conditions, including temperature, pH, ligand concentration and the presence of oxygen[82], bacteria might use the presence of these interbacterial signals to assay the growth permissiveness of the environment. Alternatively, one way that these signals could reflect the presence of other metabolically active bacteria is through the action of secreted proteins that degrade quorum sensing molecules such as acyl-homoserine lactones and thereby 'quench' the cell density signal[83].

In hosts, especially on mucosal surfaces, bacteria exist in mixed communities, and these interactions might facilitate the pathogenesis of one particular species. Of particular relevance is the formation of *S. aureus* SCVs, a persistent and dormant cell type. Prolonged culture of *S. aureus* with *P. aeruginosa* selects for SCVs, probably owing to the *P. aeruginosa* exoproduct 4-hydroxy-2-heptylquinoline-*N*-oxide, and this interaction might facilitate the survival of *S. aureus* during infections in patients with cystic fibrosis who are chronically colonized with *P. aeruginosa*[84].

As another example, an otherwise 'uncultivable' strain of *Psychrobacter* is stimulated to grow by co-culturing with a

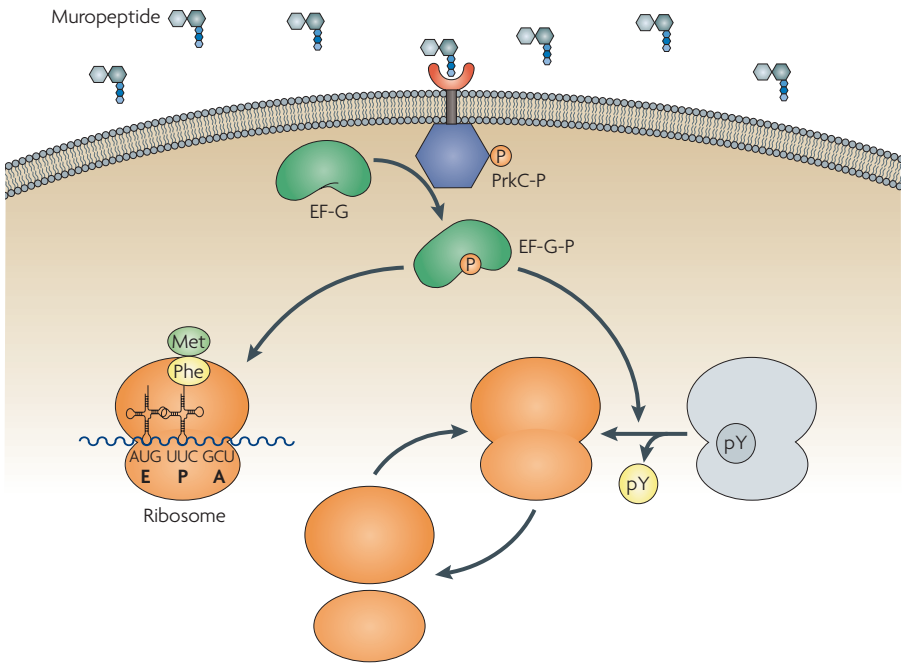

Figure 3 | **Mechanism of translation stimulation by muropeptide-induced phosphorylation of elongation factor G.** The eukaryotic-like membrane serine/threonine kinase PrkC is activated in response to muropeptide binding to its extracellular domain. PrkC-mediated phosphorylation of elongation factor G (EF-G) modulates the activity of the ribosome, initiating translation and thereby inducing exit from dormancy. Phosphorylated EF-G may also stimulate translation by blocking the action of bacterial protein Y (pY) family proteins, which prevent the disassembly of the 70S ribosome complex that is required for the ribosome to enter a new round of translation.

Cellulophaga lytica strain, and this stimulation was attributed to the production, at nanomolar concentrations, of a five amino acid peptide by the *C. lytica* 'helper' (REF. 85). As this peptide acts at a concentration far below that needed for nutrient exchange (or syntrophy), it is likely to function as a signalling molecule.

Antimicrobials. Many bacteria secrete molecules with antimicrobial properties into the environment. This release can be considered a method of bacterial communication, especially as these molecules can modulate global gene expression at subinhibitory concentrations that are more reflective of the actual concentrations of the compounds in nature[86]. As discussed above, *B. subtilis* spore germination in response to peptidoglycan is dependent on PrkC. Bryostatin, a molecule that is produced by an uncultivated marine bacterium[87] and that activates related eukaryotic serine/threonine kinases, also stimulates germination of *B. subtilis* spores in a manner dependent on the presence of the kinase[15]. Conversely, *Streptomyces staurosporeus* (also known as *Lentzea albida*) produces a small molecule called staurosporine, a well-known inhibitor of eukaryotic serine/threonine kinases that has been shown to block muropeptide-mediated spore germination at picomolar concentrations[15]. As *S. staurosporeus* is a soil bacterium, like *Bacillus* spp., this effect could be an example of the activity of an intermicrobial signalling compound. One exciting possibility is that such compounds could be used as novel antimicrobials against dormant or metabolically inactive species that are phenotypically resistant to conventional antimicrobials. Thus, in polymicrobial communities, *Streptomyces* spp. might acquire a growth advantage by preventing the transition of spores (and perhaps other dormant cell types) into growing states.

Metabolites. Many compounds released by growing cells serve as necessary metabolites for growth but could also stimulate neighbouring species to exit from dormancy. This syntrophy is common in the bacterial world[88]. For example, in a recent study the factors produced by *M. luteus* that enabled the growth of the previously uncultured species *Maribacter polysiphoniae* were identified as acyl-desferrioxamine siderophores that can bind insoluble iron[89]. In this case, the presence of siderophores reflects the activity of other bacterial species and, therefore, the ability of the environment to support microbial growth.

Conclusions and future prospects

Many pathogenic bacteria exist in dormant states that are essential for their ability to cause disease, both because these dormant cells are the infectious agents for many species and because they exhibit greater resistance to host antimicrobial factors. However, successful infections require exit from dormancy. Here, we propose that molecules released by growing bacteria, specifically cell wall muropeptides, serve as a 'wake-up call' and stimulate the growth of dormant cells through modification of the translation apparatus (FIG. 3).

Several issues are as-yet unresolved and will be the subject of future investigations. First, the kinase that detects muropeptides is well conserved, but some species contain kinases that respond to both L-Lys-containing and m-DAP-containing muropeptides (FIG. 1), whereas others respond to only m-DAP-containing muropeptides. Is this difference relevant to the microorganisms with which a particular species coexists? That is, is this difference a way of tuning the response by preventing some kinds of interspecies signalling? Second, homologues of this kinase that contain the peptidoglycan-binding domain are found in only Gram-positive species. Is this due to the muropeptide recycling pathways that are found in only Gram-negative species, which greatly reduce the release of muropeptides into the extracellular milieu? Or is it due to the presence of the outer membrane or to the much thinner peptidoglycan in Gram-negative species? Finally, what is the role of this pathway during growth? PrkC is expressed during growth[73], and its homologues have a broad but not well-understood role in bacterial physiology. Given the inability of 1,6-anhydro-muropeptides produced during stationary phase to stimulate germination, one possibility is that PrkC and its homologues act as quorum sensing systems used by bacteria to assay the number of growing bacterial cells in the milieu and to therefore regulate their own growth. However, this speculation needs to be examined carefully to distinguish homotypic (within-species) versus heterotypic (between-species) signalling.

The observation that most bacterial species are not cultivatable – 'The Great Plate Count Anomaly' (REF. 90) — has typically been attributed to non-permissive or inappropriate growth conditions, such as medium toxicity, an inappropriate incubation time or deficiencies in nutrient composition[91]. However, it has also been suggested that uncultivable microorganisms may

require signals generated by neighbouring cells[92], including growing cells[85], and, as demonstrated using dormant spore germination, these signals could include muropeptides and small-molecule kinase activators.

Jonathan Dworkin and Ishita M. Shah are at the Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA.
Correspondence to J.D.
e-mail: jonathan.dworkin@columbia.edu

doi:10.1038/nrmicro2453
Published online 25 October 2010

1. Oliver, J. D. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiol. Rev.* **34**, 415–425 (2010).
2. Jones, S. E. & Lennon, J. T. Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl Acad. Sci. USA* **107**, 5881–5886 (2010).
3. Gilbert, P., Collier, P. J. & Brown, M. R. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. *Antimicrob. Agents Chemother.* **34**, 1865–1868 (1990).
4. Domingue, G. J. Sr & Woody, H. B. Bacterial persistence and expression of disease. *Clin. Microbiol. Rev.* **10**, 320–344 (1997).
5. Oliver, J. D. The viable but nonculturable state in bacteria. *J. Microbiol.* **43**, 93–100 (2005).
6. Stephenson, K. & Lewis, R. J. Molecular insights into the initiation of sporulation in Gram-positive bacteria: new technologies for an old phenomenon. *FEMS Microbiol. Rev.* **29**, 281–301 (2005).
7. Barry, C. E. 3rd *et al.* The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nature Rev. Microbiol.* **7**, 845–855 (2009).
8. Keep, N. H., Ward, J. M., Cohen-Gonsaud, M. & Henderson, B. Wake up! Peptidoglycan lysis and bacterial non-growth states. *Trends Microbiol.* **14**, 271–276 (2006).
9. Hoare, A., Timms, P., Bavoil, P. M. & Wilson, D. P. Spatial constraints within the chlamydial host cell inclusion predict interrupted development and persistence. *BMC Microbiol.* **8**, 5 (2008).
10. Roszak, D. B. & Colwell, R. R. Survival strategies of bacteria in the natural environment. *Microbiol. Rev.* **51**, 365–379 (1987).
11. Bunker, S. T., Bates, T. C. & Oliver, J. D. Effects of temperature on detection of plasmid or chromosomally encoded *gfp*- and *lux*-labeled *Pseudomonas fluorescens* in soil. *Environ. Biosafety Res.* **3**, 83–90 (2004).
12. Oliver, J. D., Hite, F., McDougald, D., Andon, N. L. & Simpson, L. M. Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment. *Appl. Environ. Microbiol.* **61**, 2624–2630 (1995).
13. Votyakova, T. V., Kaprelyants, A. S. & Kell, D. B. Influence of viable cells on the resuscitation of dormant cells in *Micrococcus luteus* cultures held in an extended stationary phase: the population effect. *Appl. Environ. Microbiol.* **60**, 3284–3291 (1994).
14. Kaprelyants, A. S. & Kell, D. B. Do bacteria need to communicate with each other for growth? *Trends Microbiol.* **4**, 237–242 (1996).
15. Shah, I. M., Laaberki, M. H., Popham, D. L. & Dworkin, J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486–496 (2008).
16. Sunde, E. P., Setlow, P., Hederstedt, L. & Halle, B. The physical state of water in bacterial spores. *Proc. Natl Acad. Sci. USA* **106**, 19334–19339 (2009).
17. Cowan, A. E. *et al.* Lipids in the inner membrane of dormant spores of *Bacillus* species are largely immobile. *Proc. Natl Acad. Sci. USA* **101**, 7733–7738 (2004).
18. Setlow, P. I will survive: DNA protection in bacterial spores. *Trends Microbiol.* **15**, 172–180 (2007).
19. Ghosh, J. *et al.* Sporulation in mycobacteria. *Proc. Natl Acad. Sci. USA* **106**, 10781–10786 (2009).
20. Traag, B. A. *et al.* Do mycobacteria produce endospores? *Proc. Natl Acad. Sci. USA* **107**, 878–881 (2010).
21. Abdelrahman, Y. M. & Belland, R. J. The chlamydial developmental cycle. *FEMS Microbiol. Rev.* **29**, 949–959 (2005).
22. Hogan, R. J., Mathews, S. A., Mukhopadhyay, S., Summersgill, J. T. & Timms, P. Chlamydial persistence: beyond the biphasic paradigm. *Infect. Immun.* **72**, 1843–1855 (2004).
23. Harper, A., Pogson, C. I., Jones, M. L. & Pearce, J. H. Chlamydial development is adversely affected by minor changes in amino acid supply, blood plasma amino acid levels, and glucose deprivation. *Infect. Immun.* **68**, 1457–1464 (2000).
24. McCaul, T. F. & Williams, J. C. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. *J. Bacteriol.* **147**, 1063–1076 (1981).
25. Omsland, A. *et al.* Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. *Proc. Natl Acad. Sci. USA* **106**, 4430–4434 (2009).
26. Brorson, O. *et al.* Destruction of spirochete *Borrelia burgdorferi* round-body propagules (RBs) by the antibiotic tigecycline. *Proc. Natl Acad. Sci. USA* **106**, 18656–18661 (2009).
27. Kana, B. D. *et al.* The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth *in vitro*. *Mol. Microbiol.* **67**, 672–684 (2008).
28. Downing, K. J. *et al.* Mutants of *Mycobacterium tuberculosis* lacking three of the five *rpf*-like genes are defective for growth *in vivo* and for resuscitation *in vitro*. *Infect. Immun.* **73**, 3038–3043 (2005).
29. Kana, B. D. & Mizrahi, V. Resuscitation-promoting factors as lytic enzymes for bacterial growth and signaling. *FEMS Immunol. Med. Microbiol.* **58**, 39–50 (2010).
30. Hayes, C. S. & Low, D. A. Signals of growth regulation in bacteria. *Curr. Opin. Microbiol.* **12**, 667–673 (2009).
31. Apel, D., White, A. P., Grassl, G. A., Finlay, B. B. & Surette, M. G. Long-term survival of *Salmonella enterica* serovar Typhimurium reveals an infectious state that is underrepresented on laboratory media containing bile salts. *Appl. Environ. Microbiol.* **75**, 4923–4925 (2009).
32. Faulkner, G. & Garduno, R. A. Ultrastructural analysis of differentiation in *Legionella pneumophila*. *J. Bacteriol.* **184**, 7025–7041 (2002).
33. Steinert, M., Emody, L., Amann, R. & Hacker, J. Resuscitation of viable but nonculturable *Legionella pneumophila* Philadelphia JR32 by *Acanthamoeba castellanii*. *Appl. Environ. Microbiol.* **63**, 2047–2053 (1997).
34. Faulkner, G., Berk, S. G., Garduno, E., Ortiz-Jimenez, M. A. & Garduno, R. A. Passage through *Tetrahymena tropicalis* triggers a rapid morphological differentiation in *Legionella pneumophila*. *J. Bacteriol.* **190**, 7728–7738 (2008).
35. Mukamolova, G. V., Yanopolskaya, N. D., Kell, D. B. & Kaprelyants, A. S. On resuscitation from the dormant state of *Micrococcus luteus*. *Antonie Van Leeuwenhoek* **73**, 237–243 (1998).
36. Kell, D. B., Kaprelyants, A. S., Weichart, D. H., Harwood, C. R. & Barer, M. R. Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. *Antonie Van Leeuwenhoek* **73**, 169–187 (1998).
37. Lewis, K. Persister cells, dormancy and infectious disease. *Nature Rev. Microbiol.* **5**, 48–56 (2007).
38. Proctor, R. A. *et al.* Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nature Rev. Microbiol.* **4**, 295–305 (2006).
39. Schumacher, M. A. *et al.* Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. *Science* **323**, 396–401 (2009).
40. Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. Bacterial persistence as a phenotypic switch. *Science* **305**, 1622–1625 (2004).
41. Setlow, P. Spore germination. *Curr. Opin. Microbiol.* **6**, 550–556 (2003).
42. Sorg, J. A. & Sonenshein, A. L. Bile salts and glycine as cogerminants for *Clostridium difficile* spores. *J. Bacteriol.* **190**, 2505–2512 (2008).
43. Giel, J. L., Sorg, J. A., Sonenshein, A. L. & Zhu, J. Metabolism of bile salts in mice influences spore germination in *Clostridium difficile*. *PLoS ONE* **5**, e8740 (2010).
44. Doyle, R. J., Chaloupka, J. & Vinter, V. Turnover of cell walls in microorganisms. *Microbiol. Rev.* **52**, 554–567 (1988).
45. Hasegawa, M. *et al.* Differential release and distribution of Nod1 and Nod2 immunostimulatory molecules among bacterial species and environments. *J. Biol. Chem.* **281**, 29054–29063 (2006).
46. Nigro, G. *et al.* Muramylpeptide shedding modulates cell sensing of *Shigella flexneri*. *Cell. Microbiol.* **10**, 682–695 (2008).
47. Cloud-Hansen, K. A. *et al.* Breaching the great wall: peptidoglycan and microbial interactions. *Nature Rev. Microbiol.* **4**, 710–716 (2006).
48. Popham, D. L. Specialized peptidoglycan of the bacterial endospore: the inner wall of the lockbox. *Cell. Mol. Life Sci.* **59**, 426–433 (2002).
49. Gaidenko, T. A., Kim, T. J. & Price, C. W. The PrpC serine-threonine phosphatase and PrkC kinase have opposing physiological roles in stationary-phase *Bacillus subtilis* cells. *J. Bacteriol.* **184**, 6109–6114 (2002).
50. Macek, B. *et al.* Phosphoproteome analysis of *E. coli* reveals evolutionary conservation of bacterial Ser/Thr/Tyr phosphorylation. *Mol. Cell. Proteomics* **7**, 299–307 (2008).
51. Chambon, P., Deutscher, M. P. & Kornberg, A. Biochemical studies of bacterial sporulation and germination. X. Ribosomes and nucleic acids of vegetative cells and spores of *Bacillus megaterium*. *J. Biol. Chem.* **243**, 5110–5116 (1968).
52. Belland, R. J. *et al.* Genomic transcriptional profiling of the developmental cycle of *Chlamydia trachomatis*. *Proc. Natl Acad. Sci. USA* **100**, 8478–8483 (2003).
53. Bettegowda, C. *et al.* The genome and transcriptomes of the anti-tumor agent *Clostridium novyi*-NT. *Nature Biotech.* **24**, 1573–1580 (2006).
54. Gao, N., Zavialov, A. V., Ehrenberg, M. & Frank, J. Specific interaction between EF-G and RRF and its implication for GTP-dependent ribosome splitting into subunits. *J. Mol. Biol.* **374**, 1345–1358 (2007).
55. Savelsbergh, A., Rodnina, M. V. & Wintermeyer, W. Distinct functions of elongation factor G in ribosome recycling and translocation. *RNA* **15**, 772–780 (2009).
56. Janosi, L. *et al.* Evidence for *in vivo* ribosome recycling, the fourth step in protein biosynthesis. *EMBO J.* **17**, 1141–1151 (1998).
57. Vila-Sanjurjo, A., Schuwirth, B. S., Hau, C. W. & Cate, J. H. Structural basis for the control of translation initiation during stress. *Nature Struct. Mol. Biol.* **11**, 1054–1059 (2004).
58. Agafonov, D. E., Kolb, V. A. & Spirin, A. S. Ribosome-associated protein that inhibits translation at the aminoacyl-tRNA binding stage. *EMBO Rep.* **2**, 399–402 (2001).
59. Sharma, M. R. *et al.* PSRP1 is not a ribosomal protein, but a ribosome-binding factor that is recycled by the ribosome-recycling factor (RRF) and elongation factor G (EF-G). *J. Biol. Chem.* **285**, 4006–4014 (2010).
60. Jones, G. & Dyson, P. Evolution of transmembrane protein kinases implicated in coordinating remodeling of gram-positive peptidoglycan: inside versus outside. *J. Bacteriol.* **188**, 7470–7476 (2006).
61. Donat, S. *et al.* Transcriptome and functional analysis of the eukaryotic-type ser/thr kinase PknB in *Staphylococcus aureus*. *J. Bacteriol.* **191**, 4056–4069 (2009).
62. Beltramini, A. M., Mukhopadhyay, C. D. & Pancholi, V. Modulation of cell wall structure and antimicrobial susceptibility by a *Staphylococcus aureus* eukaryote-like serine/threonine kinase and phosphatase. *Infect. Immun.* **77**, 1406–1416 (2009).
63. Banu, L. D. *et al.* The *Streptococcus mutans* serine/threonine kinase, PknB, regulates competence development, bacteriocin production, and cell wall metabolism. *Infect. Immun.* **78**, 2209–2220 (2010).
64. Hett, E. C. & Rubin, E. J. Bacterial growth and cell division: a mycobacterial perspective. *Microbiol. Mol. Biol. Rev.* **72**, 126–156 (2008).
65. McCoy, A. J. & Maurelli, A. T. Building the invisible wall: updating the chlamydial peptidoglycan anomaly. *Trends Microbiol.* **14**, 70–77 (2006).
66. Mukamolova, G. V., Kaprelyants, A. S., Young, D. I., Young, M. & Kell, D. B. A bacterial cytokine. *Proc. Natl Acad. Sci. USA* **95**, 8916–8921 (1998).
67. Mukamolova, G. V. *et al.* The *rpf* gene of *Micrococcus luteus* encodes an essential secreted growth factor. *Mol. Microbiol.* **46**, 611–621 (2002).
68. Mukamolova, G. V. *et al.* Muralytic activity of *Micrococcus luteus* Rpf and its relationship to physiological activity in promoting bacterial growth and resuscitation. *Mol. Microbiol.* **59**, 84–98 (2006).

69. Shleeva, M. O. *et al.* Formation and resuscitation of "non-culturable" cells of *Rhodococcus rhodochrous* and *Mycobacterium tuberculosis* in prolonged stationary phase. *Microbiology* **148**, 1581–1591 (2002).
70. Russell-Goldman, E., Xu, J., Wang, X., Chan, J. & Tufariello, J. M. A *Mycobacterium tuberculosis* Rpf double-knockout strain exhibits profound defects in reactivation from chronic tuberculosis and innate immunity phenotypes. *Infect. Immun.* **76**, 4269–4281 (2008).
71. Hett, E. C. *et al.* A partner for the resuscitation-promoting factors of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **66**, 658–668 (2007).
72. Hett, E. C., Chao, M. C., Deng, L. L. & Rubin, E. J. A mycobacterial enzyme essential for cell division synergizes with resuscitation-promoting factor. *PLoS Pathog.* **4**, e1000001 (2008).
73. Shah, I. M. & Dworkin, J. Induction and regulation of a secreted peptidoglycan hydrolase by a membrane Ser/Thr kinase that detects muropeptides. *Mol. Microbiol.* **75**, 1232–1245 (2010).
74. Gupta, M., Sajid, A., Arora, G., Tandon, V. & Singh, Y. Forkhead-associated domain-containing protein Rv0019c and polyketide-associated protein PapA5, from substrates of serine/threonine protein kinase PknB to interacting proteins of *Mycobacterium tuberculosis*. *J. Biol. Chem.* **284**, 34723–34734 (2009).
75. Dasgupta, A., Datta, P., Kundu, M. & Basu, J. The serine/threonine kinase PknB of *Mycobacterium tuberculosis* phosphorylates PBPA, a penicillin-binding protein required for cell division. *Microbiology* **152**, 493–504 (2006).
76. Parikh, A., Verma, S. K., Khan, S., Prakash, B. & Nandicoori, V. K. PknB-mediated phosphorylation of a novel substrate, *N*-acetylglucosamine-1-phosphate uridyltransferase, modulates its acetyltransferase activity. *J. Mol. Biol.* **386**, 451–464 (2009).
77. Molle, V. & Kremer, L. Division and cell envelope regulation by Ser/Thr phosphorylation: *Mycobacterium* shows the way. *Mol. Microbiol.* **75**, 1064–1077 (2010).
78. Pietack, N. *et al. In vitro* phosphorylation of key metabolic enzymes from *Bacillus subtilis*: PrkC phosphorylates enzymes from different branches of basic metabolism. *J. Mol. Microbiol. Biotechnol.* **18**, 129–140 (2010).
79. Keller, L. & Surette, M. G. Communication in bacteria: an ecological and evolutionary perspective. *Nature Rev. Microbiol.* **4**, 249–258 (2006).
80. Shank, E. A. & Kolter, R. New developments in microbial interspecies signaling. *Curr. Opin. Microbiol.* **12**, 205–214 (2009).
81. Bassler, B. L. & Losick, R. Bacterially speaking. *Cell* **125**, 237–246 (2006).
82. Roux, A., Payne, S. M. & Gilmore, M. S. Microbial telesensing: probing the environment for friends, foes, and food. *Cell Host Microbe* **6**, 115–124 (2009).
83. Dong, Y. H. *et al.* Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* **411**, 813–817 (2001).
84. Hoffman, L. R. *et al.* Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **103**, 19890–19895 (2006).
85. Nichols, D. *et al.* Short peptide induces an "uncultivable" microorganism to grow *in vitro*. *Appl. Environ. Microbiol.* **74**, 4889–4897 (2008).
86. Yim, G., Wang, H. H. & Davies, J. Antibiotics as signalling molecules. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **362**, 1195–1200 (2007).
87. Hildebrand, M. *et al. bryA*: an unusual modular polyketide synthase gene from the uncultivated bacterial symbiont of the marine bryozoan *Bugula neritina*. *Chem. Biol.* **11**, 1543–1552 (2004).
88. McInerney, M. J. *et al.* Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Ann. NY Acad. Sci.* **1125**, 58–72 (2008).
89. D'Onofrio, A. *et al.* Siderophores from neighboring organisms promote the growth of uncultured bacteria. *Chem. Biol.* **17**, 254–264 (2010).
90. Staley, J. T. & Konopka, A. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu. Rev. Microbiol.* **39**, 321–346 (1985).
91. Leadbetter, J. R. Cultivation of recalcitrant microbes: cells are alive, well and revealing their secrets in the 21st century laboratory. *Curr. Opin. Microbiol.* **6**, 274–281 (2003).
92. Kaeberlein, T., Lewis, K. & Epstein, S. S. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* **296**, 1127–1129 (2002).
93. Aimanianda, V. *et al.* Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* **460**, 1117–1121 (2009).
94. Velagapudi, R., Hsueh, Y. P., Geunes-Boyer, S., Wright, J. R. & Heitman, J. Spores as infectious propagules of *Cryptococcus neoformans*. *Infect. Immun.* **77**, 4345–4355 (2009).
95. Osherov, N. & May, G. S. The molecular mechanisms of conidial germination. *FEMS Microbiol. Lett.* **199**, 153–160 (2001).
96. Gray, J. V. *et al.* "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **68**, 187–206 (2004).
97. Joseph-Strauss, D., Zenvirth, D., Simchen, G. & Barkai, N. Spore germination in *Saccharomyces cerevisiae*: global gene expression patterns and cell cycle landmarks. *Genome Biol.* **8**, R241 (2007).
98. Coluccio, A. E., Rodriguez, R. K., Kernan, M. J. & Neiman, A. M. The yeast spore wall enables spores to survive passage through the digestive tract of *Drosophila*. *PLoS ONE* **3**, e2873 (2008).
99. Lamarre, C. *et al.* Transcriptomic analysis of the exit from dormancy of *Aspergillus fumigatus* conidia. *BMC Genomics* **9**, 417 (2008).
100. Kasuga, T. *et al.* Long-oligomer microarray profiling in *Neurospora crassa* reveals the transcriptional program underlying biochemical and physiological events of conidial germination. *Nucleic Acids Res.* **33**, 6469–6485 (2005).
101. Giles, S. S., Dagenais, T. R., Botts, M. R., Keller, N. P. & Hull, C. M. Elucidating the pathogenesis of spores from the human fungal pathogen *Cryptococcus neoformans*. *Infect. Immun.* **77**, 3491–3500 (2009).
102. Huang, H. *et al.* Distinct patterns of dendritic cell cytokine release stimulated by fungal β-glucans and Toll-like receptor agonists. *Infect. Immun.* **77**, 1774–1781 (2009).
103. Xu, X. L. *et al.* Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating the adenylyl cyclase Cyr1p. *Cell Host Microbe* **4**, 28–39 (2008).

Acknowledgements
We thank H. Shuman for comments on the manuscript. Work from our laboratory is supported by NIH GM81368 and by a Senior Scholar Award in Aging from the Ellison Medical Foundation to J.D.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Jonathan Dworkin's homepage: http://www.microbiology.columbia.edu/faculty/dworkin.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF