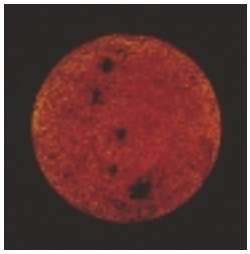
Bacterial growth in the cytosol: lessons from Listeria

septicaemia. The condition is fatal if not treated. In 1999, doctors were issued guidelines not to prescribe antibiotics for sore throats in an attempt to tackle the growing problem of antibiotic resistance. In that year, there were 37 cases of Lemierre's disease and numbers were even higher in 2001. Differential diagnosis from viral pharyngitis is by symptoms such as fever, swollen lymph glands and absence of dry cough and runny nose. *CK*
http://www.medscape.com/infectiousdiseaseshome

Broad-spectrum drugs providing diminishing returns

In the past ten years, the effectiveness of fluoroquinones against *Pseudomonas aeruginosa* has decreased >30%. Ciprofloxacin and other fluoroquinones are the only antibiotics that cystic fibrosis patients can take orally to combat *P. aeruginosa* infection. The effectiveness of these broad-spectrum antibiotics against *Escherichia coli* has also dropped. A study by the University of California, San Francisco, reveals that hospitals prescribe fluoroquinones to 30–35% of patients with streptococcal pneumonia when they are discharged, instead of penicillin. The investigators caution that doctors should routinely prescribe the narrowest-spectrum antibiotic appropriate. *AV*
http://www.eurekalert.com

Mars origin for 'superbug'?



The super-bacterium *Deinococcus radiodurans*, which can withstand radiation doses of several thousand times the lethal dose for humans, might have evolved its resistance properties on Mars. This is the exciting conclusion by a group of scientists from the Ioffe Physico-Technical Institute in St Petersburg, Russia. Based on a study of induced resistance to gamma radiation in *Escherichia coli*, the Russian scientists predicted that there has not been enough time for *Deinococcus* to have developed its remarkable radioresistance on Earth. However, the Russians have calculated that *D. radiodurans* could have been exposed to the necessary radiation levels on Mars in only a few hundred thousand years. It is speculated that the bacterium might have made the journey to Earth on rock fragments blasted from the red planet by a meteorite impact. *CK*
http://www.newscientist.com/news

N-9 and HIV risk

The spermicidal gel nonoxynol-9 (N-9), which was thought to confer protection against HIV infection, could turn out to have quite the reverse effect. This is the alarming finding of Lut Van Damme and her research team at the Institute of Tropical Medicine, Antwerp, Belgium. In a randomised trial of 765 female prostitutes in South Africa, Côte d'Ivoire, Benin and Thailand, around a third of whom used N-9 frequently, there was a doubling of the HIV-1 infection rate compared with women using a placebo gel. There was no difference in the incidence of other sexually transmitted diseases between the two groups. The study showed that frequent use of N-9 causes lesions in the vaginal wall, thereby allowing transmission of HIV. *CK*
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In Brief compiled by Cathel Kerr (clkerr@btopenworld.com) and Alexandra Venter (alexventer@hotmail.com)

Letters

Bacterial growth in the cytosol: lessons from *Listeria*

I read with interest the article in *Trends in Microbiology* by O'Riordan and Portnoy addressing the issue of whether the mammalian cytosol is permissive for bacterial growth [1]. This question was raised by a recent paper reporting that only bacteria that naturally replicate in the cytosol can grow in this host compartment following direct microinjection [2]. Previous experimental evidence, however, suggested that the cytosol is a nutrient-rich environment that can support the growth of any bacterium [1]. Observations made with *Listeria monocytogenes* might shed some light on the characteristics of the host cytosol as a replication niche and on microbial adaptations for cytosolic growth.

Unlike intracellular pathogens that proliferate in an acidic vacuole (e.g. *Salmonella enterica*), the replication of *L. monocytogenes* in the cytosol does not seem to involve the induction of stress proteins [3,4]. Although *L. monocytogenes* apparently replicates in a non-aggressive environment, its maximal generation time is longer in intracellular conditions than in rich medium (~30 min during exponential growth in brain–heart infusion vs ~60 min in J774 macrophages and ~80 min in epithelial cells and hepatocytes) (J.A. Vázquez-Boland *et al.*, unpublished). This suggests that the host cytosol is only partially permissive for bacterial growth.

L. monocytogenes auxotrophic mutants requiring purines, pyrimidines or amino acids show no substantial defects in intracellular proliferation [5,6]. However, listerial metabolic genes involved in nucleotide biosynthesis and the uptake of amino acids are induced during intracellular infection [6]. In synthetic medium, purine and pyrimidine auxotrophs are unable to grow and express the targeted genes at high levels; these effects can be reversed by the addition of 0.5 mM of the corresponding nucleotides [6]. Thus, although the host cytosol provides all of the necessary nutrients, the concentrations at which some are available might be limiting, resulting in sub-optimal growth. Metabolic intermediates are often subject to 'substrate channelling', that is, they are directly transferred between the consecutive enzymes of a pathway without escaping into the bulk phase [7]. Therefore, many of the essential metabolites present in the cytosol might be 'sequestered' in functional microcompartments, making it necessary for bacteria to adopt specific strategies to facilitate the *de novo* synthesis of essential intermediates or the acquisition of nutrients from the host cytosol.

Hpt, a UhpT-related hexose phosphate transporter recently identified in pathogenic *Listeria* spp., provides clear proof that efficient replication in the cytosol requires specific microbial adaptations [8]. *hpt* mutants do not grow on hexose phosphates but can still grow on glucose or

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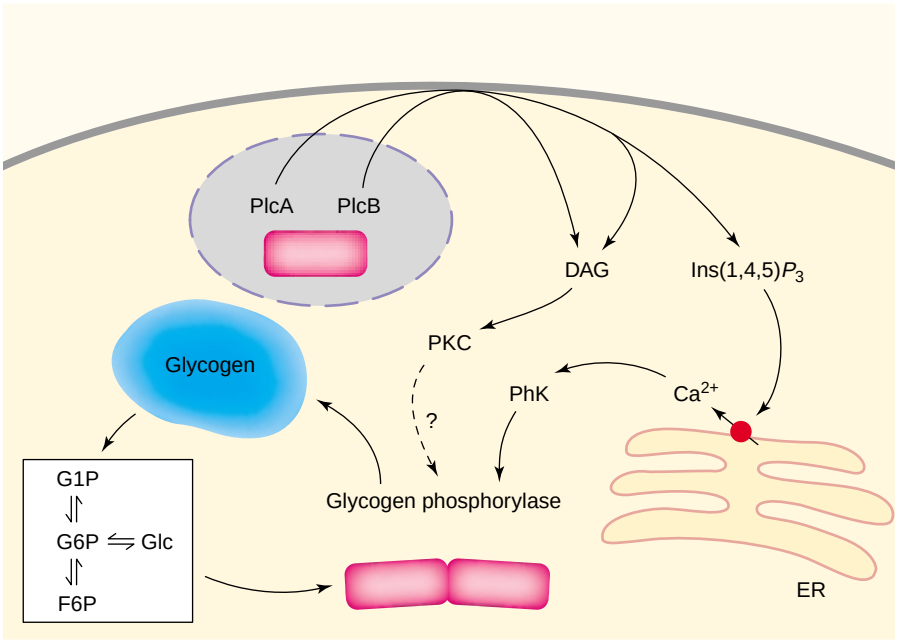

Fig. 1. Hypothetical model of listerial phospholipase C-dependent induction of glycogenolysis in infected host cells. Glycogenolysis is catalysed by glycogen phosphorylase, which is converted to its active 'a' form by phosphorylase kinase (PhK). One of the mechanisms of PhK activation involves interaction of its calmodulin-like domain with Ca^{2+}. Several glycogenolytic agents, such as adrenaline, vasopressin and eicosanoids, activate glycogen phosphorylase via a Ca^{2+}-dependent mechanism involving the mobilization of Ca^{2+} stores from the endoplasmic reticulum (ER) by inositol-1,4,5 trisphosphate [Ins(1,4,5)P_3], which is generated via hydrolysis of membrane phosphatidylinositol-4,5-bisphosphate by a phospholipase C coupled to a G protein associated with the agonist receptor. The other lipid metabolite generated by the endogenous phospholipase, diacylglycerol (DAG), activates protein kinase C (PKC), which seems to have also an important role in glycogenolysis by an as yet unknown mechanism (reviewed in [14]). *Listeria monocytogenes* induces Ca^{2+} waves in infected host cells via its phospholipases [15]. Therefore, pathogenic listeriae can potentially promote their rapid intracellular replication by actively mobilizing a sustained flux of fueling metabolites [the Hpt-transported substrates glucose-1-phosphate (G1P), glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P), as well as glucose (Glc)] to the cytosol. Interestingly, treatment of host cells with an inhibitor of glycogen phosphorylase impairs cytosolic replication of *L. monocytogenes* [16].

in rich medium. The lack of Hpt permease halves the speed of cytosolic proliferation of *L. monocytogenes* in various cell types [8], indicating that intracellular bacteria need to use not only glucose but also its phosphorylated intermediates. Thus, access to sufficient amounts of a carbon source appears to be a major limiting factor for rapid microbial proliferation in the host cytosol. Hpt is homologous to the mammalian translocase that transports glucose-6-phosphate from the cytosol into the endoplasmic reticulum in the final step of gluconeogenesis and glycogenolysis [8], illustrating that adaptation to intracellular parasitism involves mimicry of key physiological mechanisms of the eukaryotic host cell.

Hpt is tightly controlled by PrfA [8,9], the master regulator of listerial virulence. The PrfA virulence regulon is induced when bacteria enter the cytosol but is inactive in the phagosome or extracellularly [10]. Hence, although *prfA* mutants have an intact *hpt* gene, they are significantly impaired in cytosolic replication [2]. This shows that a key part of the adaptation to the cytosolic habitat is the regulatory context in which the specific nutrient-acquisition systems are integrated.

The microinjection study suggested that bacteria other than cytosolic pathogens only replicate efficiently in apoptotic cells [2]. The listerial hemolysin, listeriolysin O (LLO), mediates apoptosis [11] and incidentally the experiments supporting the notion that any bacterium that enters the cytosol can grow there were conducted using non-cytosolically replicating bacteria (e.g. *Bacillus subtilis*) that were engineered to escape the phagosome artificially by means of the membrane-disrupting functions of LLO [1,12]. Thus, the physiological state of the cell could have a major impact on the capacity of the cytosol to support bacterial growth. Interestingly, a *L. monocytogenes* deletion mutant in the phospholipase genes *plcA* and *plcB* is also significantly impaired in cytosolic replication after microinjection [2]. These listerial phospholipases induce the release of ceramide, diacylglycerol, inositol phosphates and Ca^{2+} in host cells (reviewed in [13]), second messengers involved in the control of important cellular processes. Therefore, cytosolic listeriae can take an active role in optimising their intracellular replication rate by subverting host cell signalling pathways (Fig. 1).

Finally, to explain the different results obtained by microinjection, O'Riordan and Portnoy suggest that the route of entry into the cytosol, via direct delivery or involving passage through the vacuolar compartment, might affect the ability of bacteria to grow inside host cells [1]. In invasion assays, the intracellular growth rate is calculated by counting the number of viable bacteria present in thousands of infected cells. In the microinjection study, by contrast, replication was assessed counting individual GFP-expressing bacteria under the microscope in just ten cells. These cells were pre-selected because they showed the most efficient bacterial replication, that is, about 50% of the successfully microinjected cells (which, in turn, represented 10% of all the microinjected cells) [2]. Thus, the microinjection technique might have tended to maximise differences in growth rate, perhaps to the point that bacteria with just a diminished growth capacity could have appeared to be virtually incapable of cytosolic replication. If the intracellular loads of *L. monocytogenes* and its *hpt* mutant at 8 h after infection [8] and microinjection [2] are carefully compared, it turns out that the reduction in replication efficiency observed for the *hpt* mutant is indeed quite similar for the two techniques (7.5 and 10 times, respectively). In spite of this, the *hpt* mutant, which just shows a 50% reduction in the cytosolic growth rate, was judged as being 'strongly impaired' in replication [2], or 'unable to grow significantly' [1] in the cytosol. Therefore, technical aspects and interpretation problems could also explain, at least in part, the apparent lack of consistency of the observations made via experiments using microinjection or the normal infection pathway.

In conclusion, it seems that the question is not so much whether any bacterium with access to the cytosol can multiply there, but rather whether this bacterium has evolved to multiply in that compartment efficiently.

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Published online: 11 September 2002

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Response from O'Riordan and Portnoy

We suggest that the significance of studying the capacity of the cytosol to support bacterial growth is not to debate whether cytosolic pathogens have adapted to grow efficiently in this niche, but to understand the molecular barriers to the evolution of cytosolic pathogens. If a bacterial species can access the cytosol and replicate to some extent, selection for more rapid growth can occur. By contrast, if access to the cytosol does not provide at least a minimally permissive environment, it is hard to envision how cytosolic pathogens might have evolved. Thus, the ability of the host cytosol to support some bacterial growth would probably be a prerequisite for the evolution of an intracytosolic pathogen.

Vázquez-Boland appears to be essentially in agreement with our conclusion that only specifically adapted bacteria can grow efficiently in the cytosol [1]. Control of the *Listeria monocytogenes hpt* gene by PrfA, a transcriptional regulator of *L. monocytogenes* virulence factors, is a good example of a specific bacterial adaptation to the intracellular environment. Nevertheless, a mutation in *hpt* results in only a 50% reduction in the intracellular replication rate, and bacterial numbers still increase significantly [2]. This suggests that in the absence of such a specific adaptation, the cytosol can still support significant bacterial growth, consistent with the original observation that *Bacillus subtilis* could grow in the host cytosol [3]. Furthermore, we observe that the optimal intracellular doubling time of *L. monocytogenes* in J774 macrophages (approximately 45 minutes) is very similar to that of bacteria grown in rich broth (brain–heart infusion) (M. O'Riordan and D.A. Portnoy, unpublished). This does not support the idea that the host cytosol is poor in available nutrients.

Goebel and colleagues used a novel approach to address this question, microinjecting bacteria into the host cytosol to determine its replication potential. They concluded that non-adapted bacterial species could only grow in the cytosolic environment if the host cell was undergoing apoptosis [4]. This phenomenon was proffered as an explanation for the results of multiple studies in which non-adapted bacteria did grow in the cytosol of the host cell (reviewed in [1]). It is not apparent that non-adapted bacteria, such as *B. subtilis*, engineered to access the cytosol by the expression of listeriolysin O (LLO), would cause host cell apoptosis. In the case of *L. monocytogenes*, only one cell line, derived from dendritic cells, has been shown to undergo apoptosis upon infection or upon treatment with LLO [5]. The epithelial and macrophage cell lines used in the microinjection study have not been shown to be susceptible to apoptosis by treatment with LLO or by *L. monocytogenes* infection. Indeed, macrophages do not undergo apoptotic death when infected by wild-type *L. monocytogenes* expressing LLO [6]. In addition, unlike wild-type *Salmonella typhimurium*, which normally replicate in a vacuole, an *S. typhimurium sifA* mutant can grow in the cytosol of epithelial cells [7]; in this case, no LLO is present. Therefore, we maintain that non-adapted bacteria are able to grow in the intact host cytosol, and that the apparent contradictions between published studies result from differences in technical approach and interpretation, in addition to the host cell type.

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Published online: 11 September 2002