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ANTIVIRAL IMMUNITY

Stressed mitochondria take it out on viruses

The identification of a new protein that coordinates the production of type I interferons (IFNs) in response to viral infection is reported in *Cell* this month.

The transcription factors NF- κ B and IRF3 are essential for type I IFN production in response to RNA viruses, acting downstream of the receptors TLR3 and the recently identified RIG-I. However, the mechanisms by which RIG-I activates NF- κ B and IRF3 are not clear, so the authors looked for other proteins containing caspase-recruitment domains (CARDs) similar to those of RIG-I that might be involved in this pathway. One protein with a single CARD at the amino terminus, a central proline-rich region and a hydrophobic transmembrane domain at the carboxyl terminus was identified and named mitochondrial antiviral signalling protein (MAVS) on the basis of the following properties.

Overexpression of MAVS by HEK293 cells activated a luciferase reporter under control of the IFN- α or IFN- β promoter, and MAVS was also shown to induce the production of endogenous IFN- β at both RNA and protein levels. This correlated with activation of both IRF3 and NF- κ B. When the expression of endogenous MAVS was inhibited by RNA interference (RNAi), both NF- κ B and IRF3 activation, and therefore IFN production, were abolished in response to the single-stranded RNA virus Sendai virus. The decrease in IFN production in response to viral RNA caused

by MAVS RNAi increased viral titres and the sensitivity of cells to killing by vesicular stomatitis virus, showing the physiological importance of the MAVS-mediated innate antiviral pathway.

Some of the details of this pathway were elucidated by showing that whereas RNAi of MAVS blocked IFN- β induction by RIG-I, RNAi of RIG-I did not inhibit IFN- β induction by MAVS, which indicates that MAVS lies downstream of RIG-I. Further experiments showed that MAVS lies upstream of the I κ B kinase IKK and the IRF3 kinase TBK1.

The transmembrane region of MAVS resembles that of several mitochondrial proteins, including apoptotic regulators of the BCL-2 family. Indeed, confocal microscopy and subcellular-fractionation experiments showed that MAVS co-localizes with the anti-apoptotic protein BCL-X_L in the outer mitochondrial membrane. Mutant MAVS proteins that lacked the transmembrane domain

and became cytosolic or that were targeted to different membrane locations, such as the plasma membrane or endoplasmic reticulum, had a markedly reduced ability to induce IFN- β , showing the importance of mitochondrial localization for the antiviral function of MAVS.

MAVS is therefore the first example of a mitochondrial protein with a role in innate immunity and that activates NF- κ B and IRF3. The authors suggest that mitochondria can integrate signals from cellular stresses such as viral infection — with MAVS being ideally located to detect viral replication on intracellular membranes — to induce an immune or apoptotic response depending on the challenge.

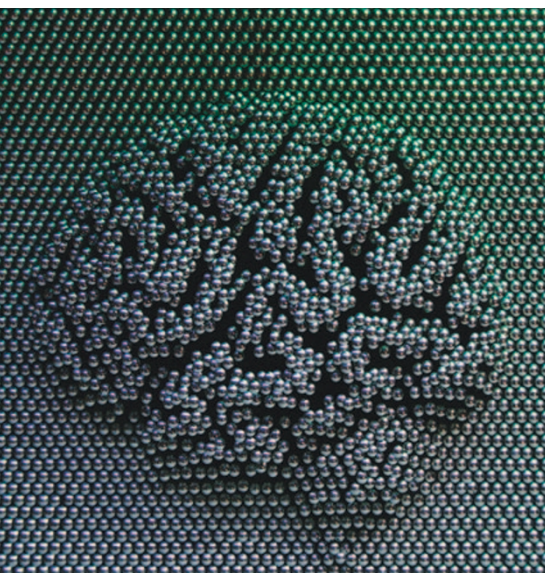
*Kirsty Minton, Associate Editor,
Nature Reviews Immunology*

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Breaching the blood–brain barrier



As the leading cause of meningitis in newborn infants, Group B *Streptococcus* (GBS) must enter the bloodstream, evade an onslaught of host defences and invade the central nervous system, an organ protected by the blood–brain barrier. A molecular understanding of how GBS achieves this final invasive step has recently been published in the *Journal of Clinical Investigation*.

The blood–brain barrier consists of a layer of specialized brain microvascular endothelial cells that adhere closely through tight junctions. Previous work revealed that GBS can efficiently invade and survive within these cells, a step that Kelly Doran and coworkers hypothesized was a prerequisite to effective penetration of the blood–brain barrier. To identify the bacterial factors responsible for invasion of brain microvascular endothelial cells, the authors generated a library of GBS

mutants that could be screened for loss of the invasive phenotype. From this analysis, a GBS gene essential to the invasion process was identified. Termed the invasion-associated gene (*iagA*), it encodes a glycosyltransferase involved in the production of a cell-membrane glycolipid anchor for lipoteichoic acid (LTA) within the cell wall of GBS. Allelic replacement of *iagA* resulted in a mutant GBS strain characterized by a 4-fold decrease in invasiveness. Doran *et al.* also confirmed that the cell membrane anchor for LTA was missing in the mutant strain and that LTA was shed into the extracellular medium.

To assess the impact of this mutation on GBS virulence *in vivo*, the authors employed a mouse model of meningitis. When the mice were challenged with the $\Delta iagA$ mutant strain, survival in the bloodstream was comparable to that observed with the wild-type strain.

Illuminating research

The versatile nature of environmental genomic surveys can be seen in two separate papers from Oded Béjà and collaborators, in which their bacterial artificial chromosome (BAC) libraries from the Mediterranean and Red Seas have been interrogated for two very different purposes.

In a study that is due to be published in the October issue of *Environmental Microbiology* and is currently available online, Zeidner *et al.* provide evidence that the phages that infect *Synechococcus* and *Prochlorococcus* could influence the evolution of the genes involved in photosynthesis in these phototrophic cyanobacteria.

In a survey of the BAC libraries using degenerate primers based on *psbA*, which encodes D1, one of the two protein components of the reaction centre of photosystem II, the authors found that the *psbA* genes present belonged to two distinct clades — a bacterial clade and a phage clade. Codon and nucleotide evolution models showed that *psbA* in the phage clade was evolving faster than in the bacterial clade, and that both clades are under similar levels of strong purifying selection.

Analyses of the GC content revealed that the phage *psbA* genes are mosaics that represent intermediates between the *psbA* genes present in *Synechococcus* and *Prochlorococcus*. Collectively, these results suggest that the *psbA* genes in cyanophage can be shuffled between phages and the two cyanobacterial genera, and this accessible phage gene pool could therefore have a crucial role in the evolution of photosynthetic gene diversity.

In a separate paper in a recent issue of *PLoS Biology*, Sabehi, Lay and others publish the results of another analysis of the same BAC libraries, this one designed to obtain a more detailed picture of the distribution of proteorhodopsin-encoding genes in the ocean photic zones.

Using new degenerate proteorhodopsin primers, the authors assessed 55 BAC clones and calculated that 13% of microorganisms in the photic zone contain proteorhodopsin genes. They went on to look for clues to other metabolic properties of proteorhodopsin-containing bacteria, and discovered that in some clones, a reverse sulphite reductase operon is present. In chemotrophs and

anaerobic phototrophs, the proteins encoded by this operon are involved in using reduced sulphur compounds as energy sources, and the authors speculate that the presence of this operon in proteorhodopsin-containing bacteria indicates that these bacteria could have a role in sulphur cycling by degrading dimethyl sulphide. Additionally, the presence of a carotenoid biosynthesis gene cluster suggests that some proteorhodopsin-containing bacteria can also synthesize retinal. The proteorhodopsin-containing bacteria are therefore more metabolically diverse than was previously suspected.

So, by interrogating the same BAC libraries with two different sets of gene primers, Béjà and collaborators have obtained a wealth of new information on what's going on beneath the waves.

Sheilagh Molloy

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ORIGINAL RESEARCH PAPERS Zeidner, G. *et al.* Potential photosynthesis gene recombination between *Prochlorococcus* and *Synechococcus* via viral intermediates. *Environ. Microbiol.* May 2005 (doi: 10.1111/j.1462-2920.2005.00833.x) | Sabehi, G. *et al.* New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *PLoS Biol.* e273 (2005)
FURTHER READING De Long, E. Microbial community genomics in the ocean. *Nature Rev. Microbiol.* 3, 459–469 (2005)

BACTERIAL PATHOGENESIS

However, virulence and the ability to cause meningitis were significantly attenuated, as evidenced by lower mortality rates (20% versus 90%) and histopathological analysis. To further dissect the $\Delta iagA$ mutant phenotype, the authors assessed the potential role of Toll-like receptor 2 and observed that attenuation of virulence in the $\Delta iagA$ mutant was independent of this host receptor. The authors also observed that soluble LTA released by the mutant GBS inhibited endothelial-cell invasion by wild-type bacteria.

Overall, the data indicate that the gene product of *iagA* contributes to the production of a glycolipid anchor for a bacterial cell-surface structure — LTA — that is crucial for GBS interaction with brain microvascular endothelial cells. This discovery of a new role for LTA in GBS pathogenesis should facilitate the development of new therapies in the ongoing fight against neonatal meningitis.

David O'Connell

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ORIGINAL RESEARCH PAPER Doran, K. S. *et al.* Blood-brain barrier invasion by group B *Streptococcus* depends upon proper cell-surface anchoring of lipoteichoic acid. *J. Clin. Invest.* **115**, 2499–2507 (2005)

Exploiting enemy measures

Switching on virulence factors at the right time and in the right place is a prerequisite for successful pathogens. However, pinpointing the signals that infecting bacteria respond to is difficult, owing to the complexity of the host environment. A new report published in *Cell* has revealed a mechanism by which bacteria switch on virulence genes in response to one component of the host innate immune armoury, the antimicrobial peptides.

A plethora of antimicrobial peptides function in host innate immunity. Although it was already known that selected antimicrobial peptides activated *Salmonella enterica* serovar *typhimurium* virulence through the PhoPQ two-component signal-transduction system, the mechanism by which the PhoQ sensor kinase detected these peptides was not clear. Antimicrobial peptides can kill pathogens by punching holes in their membranes, so it was plausible that PhoQ was sensing membrane damage rather than the antimicrobial peptide, or that another protein relayed detection of the peptide signal to the PhoQ sensor kinase.

Bader *et al.* sought to clarify how the antimicrobial peptide signal was detected, using a set of complementary approaches. Initially, experiments with a PhoQ-regulated gene fusion revealed that the periplasmic domain of the PhoQ sensor is required to respond to antimicrobial peptides. Using an *in vitro* assay, they confirmed that PhoQ alone is required to sense peptides and that peptide sensing only occurs when the peptide can contact the PhoQ periplasmic domain. This ruled out a mechanism in which PhoQ sensed membrane damage caused by the peptide. Plus, sensing peptide stimulated both the autokinase function of PhoQ and the transfer of phosphate groups from PhoQ to the PhoP response regulator protein — sensing peptides therefore stimulates the signal-transduction cascade that is required to switch on PhoPQ-regulated virulence genes.

Biophysical studies with the purified periplasmic domain of the PhoQ sensor were used to map the interaction between PhoQ and antimicrobial peptides to a negatively charged acidic surface on the protein. Presumably, non-covalent interactions occur between positively charged antimicrobial peptides and this negative patch. Divalent



cations also bound to the same surface. The mechanism favoured by the authors is that peptide binding to PhoQ disrupts cation bridges that have formed between the sensor protein and the membrane, and that this stimulates conformational changes that result in autophosphorylation of PhoQ, phosphotransfer to PhoP and activation of the cascade that ultimately results in virulence gene activation.

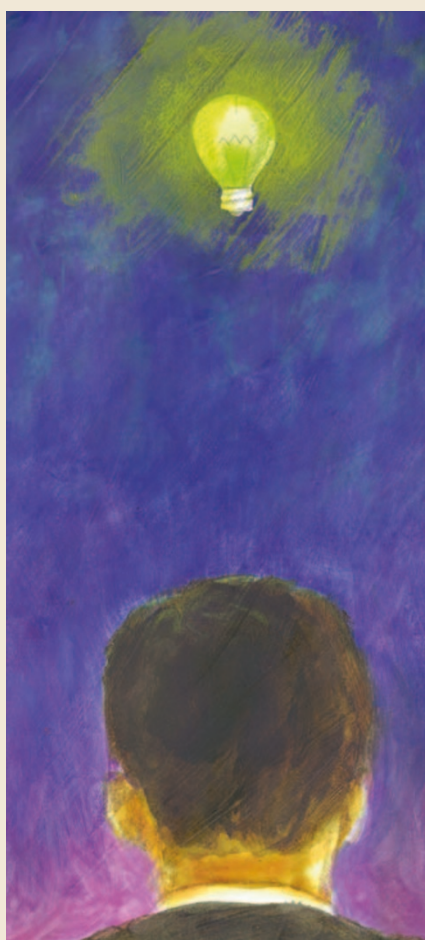
Two-component regulatory systems are present in all bacteria. While PhoPQ is essential for *S. typhimurium* virulence, the PhoPQ two-component system isn't restricted to this species and coordinates virulence-factor production in different Gram-negative pathogens that infect animals, plants and insects. So understanding how PhoPQ is activated could enable researchers to understand the pathogenic strategies of many important bacteria.

The virulence genes of *S. typhimurium* are activated after host macrophages have engulfed the invading bacterium into a phagosome. Acidification of the phagosome and production of antimicrobial peptides kills most microbial invaders, but PhoQ sensing of these signals enables the pathogen to subvert this basic host defence mechanism. As antimicrobial peptides are ubiquitous in eukaryotic hosts, subverting their function to activate virulence is another clever tactic of bacterial pathogens.

Susan Jones

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TECHNIQUES AND APPLICATIONS

Model behaviour



In the September issue of *Microbiology*, Isabel Rodríguez-Escudero and colleagues demonstrate that *Saccharomyces cerevisiae* is a suitable system for functional analysis of the virulence factors of pathogenic *Escherichia coli*.

Enteropathogenic *E. coli* (EPEC) is a major cause of diarrhoea in children, particularly

in developing countries. The bacteria adhere to intestinal enterocytes, forming attaching and effacing (A/E) lesions, and use a type III secretion system (TTSS) encoded by the locus of enterocyte effacement (LEE) to secrete effectors into host cells. In this study, the authors expressed the components of the type III secretion apparatus (EspA, EspB and EspD) and the effectors (EspF, EspG, EspH, Map and Tir) that are encoded by the LEE as fusion proteins in *S. cerevisiae*.

The formation of the A/E lesions involves reorganization of the actin cytoskeleton, and this is reflected in the yeast system — EspG disrupted cortical actin, EspF disrupted the septin rings and Map disrupted both of these cytoskeletal elements, which might indicate that it is of significant regulatory importance. Surprisingly, the translocon component EspD also disrupted actin, suggesting that it might also function as an effector protein.

Once delivered into host cells by the TTSS, the EPEC effector proteins alter host

cell signalling pathways, and this is also reflected in *S. cerevisiae*, with the expression of EspF, EspG, EspH and Map activating mitogen-activated protein kinase cascades. Perhaps most importantly, however, the yeast expression system could be used to map the functional domains of the Map effector, and it was shown that the carboxy-terminal region is necessary for actin disruption and cellular toxicity, but not for mitochondrial localization.

This work adds EPEC to the list of pathogens studied in yeast, and additionally validates this approach as an alternative method to map the functional domains of bacterial virulence factors.

Sheilagh Molloy

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MALARIA

A sweet embrace

The crystal structure of the erythrocyte-binding domain of a key malaria-vaccine candidate protein, both with and without its sugar ligand, has been published in *Cell*.



The complex malaria lifecycle includes the invasion of erythrocytes by a blood-stage parasite form, the merozoite, which results in the parasite stage that causes the onset of all the clinical symptoms of malaria. As the blood-borne merozoites are shortlived, disruption of the invasion step could control this debilitating and often fatal disease.

During invasion, the erythrocyte invasion antigen EBA-175 binds to the main erythrocyte glycoprotein, glycophorin A. The conserved RII domain of this antigen contains two Duffy-binding-like domains (DBL) that contain conserved motifs found in members of the erythrocyte-binding-like protein (EBL) superfamily. Different members of this family bind to different erythrocyte surface proteins, so that erythrocyte invasion might proceed through several different mechanisms. The EBL family is widespread among *Plasmodium* species but is restricted to this genus, so these proteins are excellent drug and vaccine targets.

Tolia *et al.* solved the crystal structure of EBA-175 RII to 2.3-Å resolution, both with and without a glycan ligand. Two monomers of RII crystallized as a dimer and were aligned antiparallel, forming two internal channels. According to the authors, this arrangement mimics a handshake. When the glycan

α -2,3-sialyllactose was cocrystallized with the RII dimer, six sugar-binding sites were identified. All six sugars were bound in the dimer interface and contacted both monomers.

The RII dimer seems to grip the sugar molecules, as four of the six sugar-binding sites are in the two channels formed by RII dimerization. The authors hypothesize that the RII protein only dimerizes when it contacts the erythrocyte surface glycoprotein glycophorin A, consistent with biochemical evidence that the predominant form of RII in solution is a monomer.

Dimerization of the extracellular RII domain of EBA-175 causes a conformational change that results in signalling by the cytoplasmic portion of the protein that in turn mediates invasion. So, the structure of the dimer could be used to inform invasion pathways that are mediated by other EBL proteins.

This novel structure will be useful to anyone wishing to model interactions involving DBL-containing proteins, and should be useful to those searching for drugs that can treat the symptoms of malaria.

Susan Jones

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ORIGINAL RESEARCH PAPER Tolia, N.H., Enemark, E.J., Sim, K.L. & Joshua-Tor, L. Structural basis for the EBA-175 erythrocyte invasion pathway of the malaria parasite *Plasmodium falciparum*. *Cell* **122**, 183–193 (2005)

IN BRIEF

PHAGE BIOLOGY

Independent virus development outside a host

Häring, M. *et al. Nature* **436**, 1101–1102 (2005)

All viruses require a host cell for functional activity, right? Wrong, according to a paper recently published in *Nature*. The authors report their discovery of an archaeal virus that develops a long tail from both ends only once it has been released from the host cell. The virus was isolated from an acidic hot spring at Pozzuoli, Italy, and was detected in enrichment culture as lemon-shaped particles bearing appendages of varying lengths at both ends. The virus, named ATV, could be cultured in the hyperthermophilic archaeon *Acidanius convivator* but, surprisingly, tails were only observed after infected cell cultures had been maintained at 75°C for 8 days. Analyses confirmed that the tail development is an active biological process. As ATV causes a lytic infection, the host-independent development of tails could be a useful strategy for survival in the external environment.

ENVIRONMENTAL MICROBIOLOGY

Computational improvements reveal great bacterial diversity and high metal toxicity in soil

Gans, J. *et al. Science* **309**, 1387–1390 (2005)

Accurate measurements of the species abundance of microorganisms in environments such as soil are extremely difficult to obtain. In a recent issue of *Science*, researchers from Los Alamos National Laboratory have published details of a new analytical approach, based on reassociation kinetics, which allows different species-abundance models to be quantitatively compared. The authors then reanalysed three reassociation data sets from a study that had assessed the impact of metal pollution on bacterial diversity and abundance. The analysis revealed that in the original study, the abundance of microorganisms had been underestimated by two orders of magnitude. The effects of metal pollution had also been underestimated — although the overall bacterial biomass was unaffected, bacterial diversity was reduced by >99.9%.

PHAGE BIOLOGY

The single-stranded genome of phage CTX is the form used for integration into the genome of *Vibrio cholerae*Val, M.-E. *et al. Mol. Cell* **19**, 559–566 (2005)

The *Vibrio cholerae* positive single-stranded (ss) DNA phage CTXΦ integrates into the *V. cholerae* genome using the host-encoded tyrosine recombinases XerC and XerD. Now, new work published in *Molecular Cell* by Val *et al.* provides more molecular details on the integration reaction. The new data reveal that folding of the phage ssDNA genome between the XerC- and XerD-binding sites into a hairpin structure creates the reaction substrate for XerCD, allowing integration into the host genome while at the same time preventing inappropriate excision. This reaction differs greatly from the classical model of ssDNA integration and creates a new paradigm for tyrosine recombinase action.



BACTERIAL PATHOGENESIS

Nosy neighbours

Mucosal surfaces are home to a large variety of common bacteria that compete with one another for the resources in this crowded habitat. Now, a new report in *PLoS Pathogens* by Jeffrey Weiser and colleagues reveals that bacteria use an ingenious tactic to outmanoeuvre their competitors — they manipulate the immune response of the host and direct it against their neighbours.

The authors used a mouse model to study the competitive interactions of two bacteria that commonly reside in the human nasopharynx — *Streptococcus pneumoniae* and *Haemophilus influenzae*. When pitted against each other in laboratory culture, *S. pneumoniae* rapidly killed *H. influenzae* by unleashing a barrage of bactericidal agents. But when these two pathogens were simultaneously introduced into the nasopharynx of mice, the outcome was entirely different — *S. pneumoniae* was rapidly cleared from the mouse upper respiratory tract, whereas *H. influenzae* was undepleted. Importantly, the air passages of animals colonized simultaneously with both bacteria were replete with neutrophils — the key inflammatory cells of the innate immune response. And the depletion of neutrophils from co-colonized mice eliminated the survival advantage of *H. influenzae*, which indicates that the innate immune response of the host is crucial to the competitive strategies of this microorganism.

However, it is not only the chemotactic powers of *H. influenzae* that determine its competitive success — *ex vivo* assays showed that components of *H. influenzae*, but not *S. pneumoniae*, efficiently activated the microbicidal activity of neutrophils. Also, neutrophils that were primed by *H. influenzae* did not adversely affect the bacterium itself, and the authors contend that it is this selective response of inflammatory cells to products from one bacterial species, but not another, that provides a mechanism to defeat competitors.

Although as yet unproven, other combinations of co-colonizing bacteria might compete with each other in similar ways. For instance, it has recently been shown that children immunized with pneumococcal vaccine have lower rates of carriage of *S. pneumoniae*, but are more likely to harbour nasal *Staphylococcus aureus* and develop staphylococcal ear infections.

These results show that upsetting the delicate balance of the mucosal microflora, for example, by using narrow-spectrum antibiotics and vaccines, might well have adverse consequences for the host, and it is therefore imperative that we understand how microbial communities interact in the living host.

Shannon Amoils

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