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## VACCINES

# Packing the punch into BCG

Infection with *Mycobacterium tuberculosis* remains a leading cause of death worldwide, despite the widespread use of the *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccine. Although BCG provides protection against disseminated childhood tuberculosis, protection against pulmonary tuberculosis in adults is limited, in part because of its inefficient activation of T-cell immunity.

Now, reporting in the *Journal of Clinical Investigation*, Stefan Kaufmann and colleagues describe the generation of a novel BCG strain equipped with a virulence protein from *Listeria monocytogenes*. The authors provide evidence for the increased protective effect of this vaccine and propose a mechanism to explain its enhanced efficacy.

Protection against *M. tuberculosis* requires optimal CD4<sup>+</sup> and CD8<sup>+</sup> T-cell immunity, but it is the generation of the latter that poses a problem. Both *M. tuberculosis* and BCG reside in the phagosomes of antigen-presenting cells (mainly macrophages and dendritic cells), which channel mycobacterial antigens into the MHC class II pathway. As CD4<sup>+</sup> T cells, but not CD8<sup>+</sup> T cells, interact with MHC class II molecules, the isolation of infecting mycobacteria in phagosomes favours the activation of the helper-T-cell response.

To redress the balance of the T-cell response to mycobacterial infection, Kaufmann and colleagues generated



a recombinant BCG (rBCG) strain that secreted listeriolysin, a protein used by *L. monocytogenes* to perforate the phagosome membrane. As the ‘hole punching’ function of listeriolysin is optimal at an acid pH, the BCG urease C gene, which encodes an enzyme that neutralizes the pH of the phagosome, was also deleted. The authors reasoned that disruption of the phagosome by functional listeriolysin would dislodge BCG into the cytoplasm, activating CD8<sup>+</sup> T cells through the MHC class I pathway.

Indeed, macrophages infected with the  $\Delta ureC hly^+$  rBCG strain accumulated mycobacterial antigens in the cytoplasm. And the new vaccine strain was significantly more effective than unmodified BCG in protecting mice against infection with both a laboratory strain and a new clinical isolate of *M. tuberculosis*.

Although the enhanced protection offered by  $\Delta ureC hly^+$  rBCG might be due to increased cytoplasmic antigen loading of MHC class I molecules, experiments conducted by Kaufmann and his team suggested an additional possibility. Previous work has shown that disruption of the phagosome induces programmed cell death in infected cells, which release apoptotic blebs with mycobacterial antigen cargo. Neighbouring dendritic cells take up these apoptotic blebs and channel antigens into the MHC class I pathway, with efficient activation of CD8<sup>+</sup> T cells. It is this phenomenon, known as cross priming, that seems to be crucial for superior vaccine efficacy.

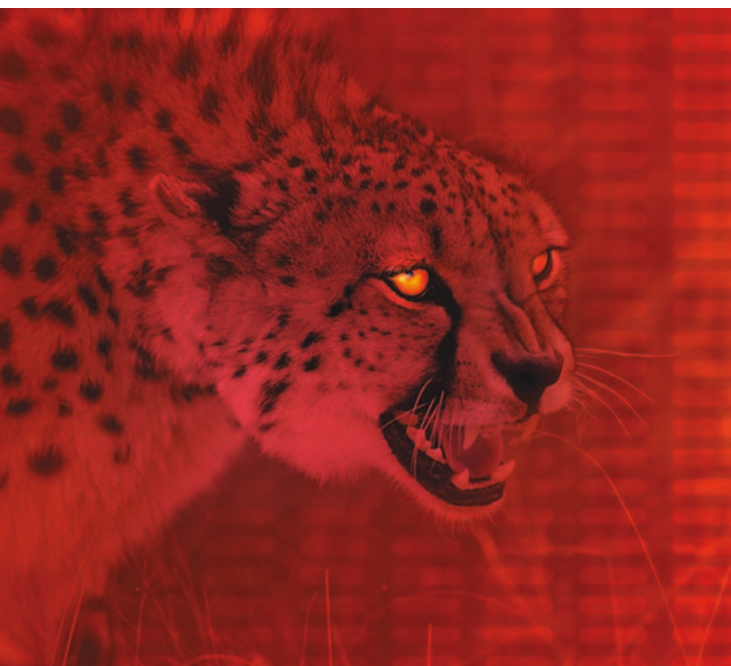
Shannon Amoils

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**ORIGINAL RESEARCH PAPER** Grode, L. *et al.* Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette–Guérin mutants that secrete listeriolysin. *J. Clin. Invest.* **9**, 2472–2479 (2005)

## BACTERIAL PHYSIOLOGY

## Bacterial fight or flight?



Several previously unknown functions of the universal stress protein A (UspA) family in *Escherichia coli* have been revealed by a new functional genomic analysis recently published in the *Journal of Bacteriology*.

It had previously been observed that the *E. coli* UspA proteins undergo coordinated induction in response to various stresses, but there was little information on the functions of individual UspAs. There are six *E. coli* UspA proteins, and they can be divided into four classes based on structural motifs — three proteins (UspA, UspC and UspD) belong to class I, two to class II (UspF and UspG) and the sixth protein, UspE, belongs to both classes III and IV. Laurence Nachin, Ulf Nannmark and Thomas Nyström used this information to create a series of chromosomal deletion mutants designed to analyse the functions of members of each class.

Analysis of growth in the presence of phenazine methosulphate revealed

that two of the three class I proteins, UspA and UspD, are involved in defence against oxidative stress. In addition, the  $\Delta uspD$  mutant showed the greatest sensitivity to the addition of streptonigrin, an antibiotic with iron-dependent activity, indicating a role for this class I protein in intracellular iron scavenging. Neither of these functions had previously been attributed to UspA proteins. A serendipitous observation revealed another previously unknown function — the authors noticed that the different mutant cultures had different flocculation profiles, with cell–cell aggregation greatly reduced in  $\Delta uspC$  and  $\Delta uspE$  cultures, indicating that these proteins might be involved in regulating adhesion capabilities.

The ability of the different mutant cultures to form fimbriae was then assessed using a yeast agglutination assay. Agglutination was enhanced in  $\Delta uspC$  and  $\Delta uspE$  cells and reduced in  $\Delta uspF$  and  $\Delta uspG$  cells. As adhesion and motility often

## VIROLOGY

## HCV's little helper

Viruses can appropriate various host-cell pathways to increase their replicative and infective abilities. Now, a report published in *Science* reveals a novel mechanism by which hepatitis C virus (HCV) increases its abundance in the cell. Catherine Jopling and colleagues show that the HCV RNA genome binds to host-cell microRNA (miRNA) to facilitate viral replication.

miRNAs are small RNA molecules that regulate genes in many plant and animal species either by promoting mRNA cleavage or by preventing mRNA translation. Whereas many miRNAs are ubiquitously expressed, others are tissue specific. And in some instances, the expression of miRNAs is limited to certain cell lines — for example, liver-specific miR-122 is expressed in the Huh7 cell line, but not in HepG2 cells, both of which are derived from human hepatocytes. The Huh7 cell line can support HCV replication, and the authors asked whether this replicative ability could be related to the expression of miR-122 in this permissive cell line.

Inspection of the HCV genome revealed two potential miR-122 binding sites, one in the viral 3' non-coding region (NCR) and the other in the viral 5' NCR. To determine whether miR-122 regulates HCV gene expression, Jopling *et al.* studied the abundance of viral RNA in Huh7 cells that stably expressed an HCV replicon. When miR-122 was inactivated by sequestration using a complementary oligonucleotide, the amount of HCV replicon RNA was reduced by ~80%. Similarly, replication-competent genomic HCV RNA accumulated in Huh7 cells only in the presence of miR-122.

By mutating the HCV genome and ectopically expressing miR-122 molecules with compensatory mutations, the authors showed that HCV RNA directly bound to miR-122 through the 5' NCR, and that this interaction increased viral RNA levels.

Notably, although most miRNAs impinge on gene expression at the level of mRNA translation, this does not seem to be the case for miR-122. Experiments using replication-deficient HCV RNAs showed

that mutations of the miR-122 binding site did not affect mRNA translation or stability. Instead, it seems that miR-122 positively influences viral replication, perhaps by aiding RNA folding or RNA accumulation into replication complexes.

This is the first example of an animal RNA that interacts with its target 5' NCR, and it will be interesting to note whether other viral 5' NCRs are similarly targeted by miRNAs. Finally, as a staggering 170 million people are currently infected with HCV, there is an urgent need for curative therapy. Based on these results, the authors hope that the targeted inactivation of miRNA-122 might prove to be effective antiviral therapy.

Shannon Amoils

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**ORIGINAL RESEARCH PAPER** Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M. & Sarnow, P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* **309**, 1577–1581 (2005)

**WEBSITE**

Peter Sarnow's laboratory:  
[http://cmgm.stanford.edu/micro/sarnow\\_lab](http://cmgm.stanford.edu/micro/sarnow_lab)

show reciprocal regulation, motility was then examined in a swimming assay, and was found to be decreased in  $\Delta uspC$  and  $\Delta uspE$  mutants and increased in  $\Delta uspF$  and  $\Delta uspG$  mutants. Further analysis revealed that  $\Delta uspC$  and  $\Delta uspE$  cells lack flagella. Therefore, the authors note that, in addition to their other newly identified roles, UspA proteins are also involved in “reprogramming the cell towards defense and escape”.

This study highlights the power of functional genomics and shows that the extensively studied model organism *E. coli* still holds some surprises. Contrary to expectation, the UspA proteins have distinct, as well as overlapping, functions, and some might be involved in controlling a bacterial version of the ‘fight or flight’ response.

Sheilagh Molloy

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**ORIGINAL RESEARCH PAPER** Nachin, L., Nannmark, U. & Nyström, T. Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion and motility. *J. Bacteriol.* **187**, 6265–6272 (2005)

#### MALARIA

## Alternative entry

*Plasmodium falciparum* causes the deadliest form of malaria, resulting in over a million deaths every year. Now, a team led by Alan F. Cowman has identified a gene that is used by this parasite to switch between the pathways it can use to invade red blood cells, and published their discovery in *Science*.

*P. falciparum* uses different pathways to invade the host erythrocyte. Some strains mainly use ligands that bind to erythrocyte receptors that contain sialic acid, whereas other strains use ligands that bind to erythrocyte receptors without sialic acid. Parasites of the W2mef *P. falciparum* strain can switch from sialic-acid-dependent to sialic-acid independent invasion. This can be achieved in the laboratory by selection on erythrocytes treated with neuraminidase, an enzyme that removes the sialic-acid residues, as well as by disruption of the sialic-acid-dependent ligand erythrocyte-binding antigen EBA175 ( $W2mef\Delta175$ ).

When selecting two clonal lines of W2mef ( $W2m/c2/N$  and  $W2m/c4/N$ ) for sialic-acid-independent invasion on neuraminidase-treated erythrocytes, parasites were obtained that showed invasion of erythrocytes comparable to that of  $W2mef\Delta175$ . When these parasites were grown on normal erythrocytes for several months, the clonal lines reverted to sialic-acid-dependent invasion. This ability to switch invasion pathways was reproducible.

To detect any shift in gene expression between W2mef,  $W2mef\Delta175$  and  $W2m/c4/N$ , Stubbs *et al.* used oligonucleotide arrays, and found that *PfRh4* (*P. falciparum* reticulocyte-binding-like homologue 4) showed reproducible transcriptional differences. Using RT-PCR, they also found that transcription of *PfRh4* increased about 60- to 80-fold in the sialic-acid-independent lines compared with the sialic-acid-dependent lines, suggesting that the activation of *PfRh4* is required for switching from sialic-acid-independent invasion to sialic-acid-independent invasion. Accordingly, *PfRh4*-specific antibodies did not detect *PfRh4* in sialic-acid-dependent parasites, whereas it was expressed in sialic-acid-independent parasites.

To confirm the role of *PfRh4* in the switch from sialic-acid-independent to sialic-acid-independent invasion, transgenic parasites in which the *PfRh4* gene was disrupted were grown on normal or neuraminidase-treated cells. These



transgenic parasites were unable to switch to sialic-acid-independent invasion, so expression of the *PfRh4* protein is required for this process. Finally, Cowman and colleagues determined the location of *PfRh4* in the parasite by constructing transgenic parasite lines that expressed *PfRh4* as a chimaeric protein with green fluorescent protein. They found that the protein is located at the apical tip of merozoites, which is consistent with a direct function of *PfRh4* in invasion of erythrocytes.

This discovery of the role of *PfRh4* in the switching of invasion strategies has important implications for anti-malaria vaccine design. A functional binding domain of the sialic-acid-dependent ligand EBA175 is currently being developed as a possible malaria vaccine. However, current efforts to target ligands that have a role in sialic-acid-dependent invasion might not be sufficient if the parasite can switch to a sialic-acid independent invasion pathway.

Annie Tremp

#### 🔗 References and links

**ORIGINAL RESEARCH PAPER** Stubbs, J. *et al.* Molecular mechanism for switching of *P. falciparum* invasion pathways into human erythrocytes. *Science* **309**, 1384–1387 (2005)  
**FURTHER READING** Touré, Y. T. & Oduola, A. Focus: malaria. *Nature Rev. Microbiol.* **2**, 276–277 (2004)



## TECHNIQUES AND APPLICATIONS

## A cautionary tale...

A short report in the October issue of *Infection and Immunity* reveals that the presence of cloning vectors or fluorescent proteins could be affecting the results of bacterial pathogenicity studies.

Leigh Knodler and colleagues wanted to test whether the presence of plasmids or fluorescent reporter proteins such as green fluorescent protein (GFP) *per se* had any effect on the ability of *Salmonella enterica* serovar Typhimurium to invade and replicate in eukaryotic host cells. To do so, they carried out a series of simple experiments involving commonly used plasmids of low-to-medium copy number carrying selectable markers.

*Salmonella* can enter cells either by an active, actin-dependent process using effectors encoded by *Salmonella* pathogenicity island 1 (SPI-1) or by SPI-1-independent phagocytosis. Cell-invasion experiments in non-phagocytic HeLa and phagocytic RAW264.7 cells demonstrated

that some plasmids could decrease the efficiency of both SPI-1-mediated and non-SPI-1-mediated uptake, as well as affecting intracellular replication. Additionally, Knodler *et al.* also found that the presence of GFP or red fluorescent protein (RFP) impaired the ability of serovar Typhimurium to invade both cell lines, and RFP also impaired serovar Typhimurium intracellular replication in HeLa cells.

As mouse models are commonly used in *Salmonella* pathogenesis studies, Knodler *et al.* went on to investigate whether the presence of plasmid pACYC184, GFP or RFP had any effects on serovar Typhimurium infection in mice. No effect was seen for pACYC184, but the presence of GFP and RFP did reduce the competitive index of the bacteria expressing these protein markers.

So, this work shows that, depending on the route of uptake, the presence of simple plasmid cloning vectors or fluorescent protein markers can perturb the interaction



of *S. enterica* with host cells. The authors conclude that “results from *in vitro* and *in vivo* experiments that rely exclusively on GFP- and RFP-expressing bacteria should be interpreted with some caution”.

Sheilagh Molloy

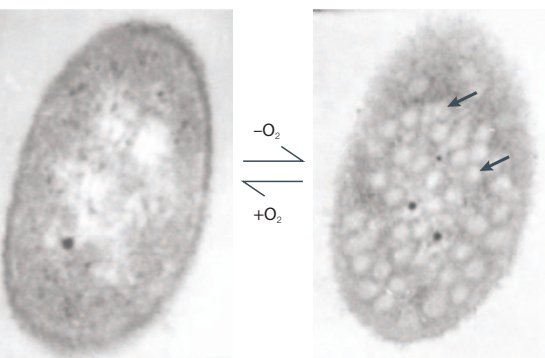
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**ORIGINAL RESEARCH PAPER** Knodler, L. A. *et al.* Cloning vectors and fluorescent proteins can significantly inhibit *Salmonella enterica* virulence in both epithelial cells and macrophages: implications for bacterial pathogenesis studies. *Infect. Immun.* **73**, 7027–7031 (2005)

## BIOINFORMATICS

## Exploiting the genome: DNA motifs

A commonly asked question among researchers analysing genomic data is, given a set of microarray experiments where the activities of different *cis*-acting regulatory proteins vary, is it possible to predict the DNA protein-binding motifs upstream of the regulated genes? A paper just published in *Microbiology* describes an approach that addresses this issue.



The transition of *Rhodospirillum rubrum* from an aerobic to anaerobic environment triggers regulatory events that result in the formation of photosynthetic membranes (indicated with arrows) under the regulatory control of PpsR, FnrL and PrrA. Image courtesy of C. Mackenzie, The University of Texas Health Science Center, USA.

In an era where huge genomic and functional genomic data sets are being generated on a daily basis, a challenge for biologists is to develop techniques that allow the extraction of useful information that can inform and guide further experimental investigation. In this study, Haluk Resat and colleagues focused on the search for DNA motifs present in the genome of the photosynthetic bacterium *Rhodospirillum rubrum* that bind three transcription factors known to regulate photosynthetic gene expression — PrrA, PpsR and FnrL. The approach used by the authors was to first perform a hierarchical clustering of *R. rubrum* genes using microarray mRNA expression data to identify genes that showed similar expression patterns under different experimental conditions. Second, the DNA sequences upstream of these genes were analysed for signature sites that suggested possible co-regulation. These sites were then used to generate predicted consensus sequences that formed the basis of a whole-genome-level search to identify putative new target genes for these regulators.

As a validation of the approach, Mao *et al.* independently identified PpsR and FnrL binding sites that were consistent with previously published consensus sequences for these transcription factors. The authors also extended the number of possible target genes regulated by these proteins. Further analysis of the PrrA DNA-binding sequence indicated that it consists of two conserved elements with a variable-sized gap in between. Last, using the three consensus sequences, a whole genome analysis of the *R. rubrum* genome revealed that the PrrA regulon was considerably larger than that of PpsR and FnrL, providing evidence that PrrA is a global regulator for gene expression in this microorganism.

The authors note that, as with all prediction techniques, the generation of false-positive and false-negative results is possible; however, the technique is sufficiently robust to assist in the useful prediction of genes regulated by these transcription factors. The approach should also be applicable to additional gene clusters derived from microarray data, and facilitate the identification of regulatory elements crucial to other biological processes.

David O'Connell

 **References and links**

**ORIGINAL RESEARCH PAPER** Mao, L. *et al.* Combining microarray and genomic data to predict DNA binding motifs. *Microbiology* **151**, 3197–3213 (2005)



## ANTIVIRAL IMMUNITY

## First line of defence?

Defensins are lectin-like antimicrobial peptides that are produced by cells in both plants and animals. They are critical for innate host defence, although the precise details of their mode of action has remained unsolved. Now, a new report published in *Nature Immunology* reveals a general mechanism of action for how defensins block influenza-virus infection.

Leikina and colleagues initially focused on the  $\theta$ -defensin retrocyclin 2 (RC2), which, along with many of the other multivalent lectins found in the innate immune system, has broad-spectrum antiviral properties. Their results demonstrated that, rather than affecting later stages of infection, RC2 in fact inhibited viral entry into the cell. The authors were able to rule out both viral binding and endocytosis as the stages of viral entry at which RC2 had an effect, leaving the final stage, membrane fusion, as the probable target.

Haemagglutinin (HA) is the receptor-binding and membrane-fusion glycoprotein of influenza virus, and is a target for neutralizing antibodies. HA undergoes conformational changes upon activation, which triggers membrane fusion. Leikina *et al.* were next able to demonstrate that, although RC2 did not prevent conformational changes in HA, it did block at least one of the other processes involved in HA-mediated membrane fusion, thereby preventing viral entry. In addition, the authors showed that RC2 is not

specific to HA, but can inhibit fusion mediated by other viral proteins.

RC2, like other lectins, binds cell-surface carbohydrates (glycans). To determine if this quality contributed to the fusion inhibition, the authors examined whether deglycosylation could abolish RC2's antiviral effect. Their positive results confirmed that RC2 interacts with carbohydrates at the cell surface, and further investigation showed that RC2 immobilized the movement of membrane glycoproteins. This led to the conclusion that RC2 crosslinks those surface proteins in order to inhibit fusion. Supporting these findings, Leikina *et al.* also demonstrated that two other lectin components of the innate immune system, mannan-binding lectin and human  $\beta$ -defensin 3, similarly inhibited the step that precedes viral entry.

Many of the antimicrobial peptides of the innate immune system possess a broad spectrum of antiviral activity; this novel mechanism of blocking viral fusion by crosslinking and immobilizing surface glycoproteins might help to explain this phenomenon, and further investigation could ultimately point to new therapeutic strategies for preventing viral illness.

Sharon Ahmad

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**ORIGINAL RESEARCH PAPER** Leikina, E. *et al.* Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoproteins. *Nature Immunol.* **6**, 995–1001 (2005)

**FURTHER READING** Lehrer, R. I. Primate defensins. *Nature Rev. Microbiol.* **2**, 727–738 (2004)

## IN BRIEF

## ENVIRONMENTAL MICROBIOLOGY

### The 'pH optimum anomaly' of intracellular enzymes of *Ferroplasma acidiphilum*

Golyshina, O. V. *et al.* *Environ. Microbiol.* September 2005 (doi:10.1111/j.1462-2920.2005.00907.x)

The metabolic adaptations of extremophiles continue to surprise. In a recent issue of *Environmental Microbiology*, Olga Golyshina *et al.* determined the pH optima of several intracellular enzymes from the archaeal acidophile *Ferroplasma acidiphilum*. Although *F. acidiphilum* thrives at pH 0–2, it maintains a near neutral cytoplasmic pH, implying that intracellular enzymes function optimally at a neutral pH. However, *in vitro* assays showed that these enzymes were stable at an acidic pH range. The authors suggest that this 'pH optimum anomaly' might be due to the seclusion of these enzymes inside acidic compartments in the cytoplasm of *F. acidiphilum*. Other alternatives are proposed, including the possibility that these enzymes function in multienzyme complexes with neutral pH optima.

## FUNGAL PATHOGENESIS

### LaeA, a regulator of morphogenetic fungal virulence factors

Bok, J.W. *et al.* *Eukaryot. Cell* **4**, 1574–1582 (2005)

The development of antifungal therapies has been hampered by the functional redundancy of fungal disease determinant genes and the troublesome side effects of potential fungicides. This study identifies LaeA — a positive regulator of secondary metabolism — as a virulence factor in the opportunistic fungus *Aspergillus fumigatus*. In a murine model of *Aspergillus* infection,  $\Delta laeA$  mutants showed reduced virulence associated with decreased levels of pulmonary gliotoxin.  $\Delta laeA$  hyphae killed fewer neutrophils than wild-type hyphae, and macrophage phagocytosis of  $\Delta laeA$  conidia was enhanced. LaeA is a conserved protein in filamentous fungi and, as its functions are fungal specific, it represents a promising non-toxic drug target.

## ARCHAEA

### Isolation of an autotrophic ammonia-oxidizing marine archaeon

Könneke, M. *et al.* *Nature* **437**, 543–546 (2005)

Although Archaea were long believed to be obligate extremophiles, it is now known that the Crenarchaeota comprise a large fraction of marine bacterioplankton. This study is the first to report the isolation of an ammonia-oxidizing chemolithotrophic marine crenarchaeote. Although this archaea was isolated from a tropical tank at the Seattle aquarium, phylogenetic analyses indicate a close relationship with the marine group 1 Crenarchaeota. This implies that nitrifying Crenarchaeota might be important contributors to global carbon and nitrogen cycles. Furthermore, this novel archaeon underscores the debate surrounding the origins of ammonia oxidation — bacterial or archaeal, mesophile or thermophile?