INNATE IMMUNITY

Finding flagellin

Bacterial flagellin is a known ligand for the Toll-like receptor TLR5. However, several recent papers have now revealed that in addition to the TLR5 pathway, which responds to extracellular flagellin, host macrophages can respond to cytosolic flagellin through members of the NOD-like receptor (NLR) family.

The recognition of pathogen-associated molecular patterns by host TLRs is a key component of innate immunity and much has been learned about TLRs and their signalling pathways over the past decade.

More recently, attention has turned to the role of non-TLRs in innate immunity, including the cytoplasmic NLR family. Details of the NLR signalling pathways are beginning to emerge and NLRs are known to be involved in secretion of the pro-inflammatory cytokine interleukin-1β (IL-1β) by macrophages. IL-1β is produced initially as a zymogen that is activated for secretion by caspase 1.

In *Salmonella typhimurium* infection, the NLR protein Ipaf was known to be involved in caspase 1 activation and IL-1β secretion but, until now, the *S. typhimurium* ligand for Ipaf was unknown. Two independent groups led by Gabriel Núñez and Alan Aderem investigated the nature of the innate immune response to *S. typhimurium* infection. Both groups confirmed that Ipaf was required for IL-1β production and caspase 1 activation in macrophages. Additionally, they both found that *S. typhimurium* mutants that either lack or have mutated flagella did not stimulate caspase 1 activation or IL-1β secretion, suggesting that flagellin is the *S. typhimurium* ligand for Ipaf.

As flagellin is also a known ligand for TLR5, the involvement of TLRs was examined — both groups found that *S. typhimurium* could stimulate caspase 1 activation and IL-1β secretion in TLR5-deficient macrophages, and in wild-type macrophages, and in addition Franchi *et al.* found normal levels of caspase 1 activation and IL-1β secretion in tolerant macrophages that are refractory to TLR stimulation. Taken together, these results suggest that macrophages sense flagellin through a TLR5-independent pathway that relies on the cytoplasmic sensor Ipaf. Further confirmation that Ipaf senses flagellin in the cytosol independently of TLR5 comes from the fact that both groups also demonstrated that purified flagellin delivered to the cytosol triggered caspase 1 activation in wild-type but not Ipaf-deficient macrophages. The mechanism by which flagellin accesses the cytosol during infection remains to be completely elucidated, however genetic evidence presented by Miao *et al.* suggests that it is transferred directly into the host cell cytoplasm by the virulence-associated type III secretion system.

These results are echoed by results published recently in two independent papers, one in *PLoS Pathogens* and one in *Journal of Experimental Medicine*, which indicate that an NLR is also involved in cytosolic sensing of *Legionella pneumophila* flagellin through a TLR5-independent, caspase-1-dependent pathway.

Sheilagh Molloy

ORIGINAL RESEARCH PAPERS Franchi, L. *et al.* Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1β in *Salmonella*-infected macrophages. *Nature Immunol.* 30 April 2006 (doi:10.1038/ni1346) | Miao, E. A. *et al.* Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. *Nature Immunol.* 30 April 2006 (doi:10.1038/ni1344) | Ren, T. *et al.* Flagellin-deficient *Legionella* mutants evade caspase 1 and *Naip5*-mediated macrophage immunity. *PLoS Pathogens* **3** e18 (2006) | Molofsky, A. B. *et al.* Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* **203**, 1093–1104 (2006)

ADAPTIVE IMMUNITY

Private repertoire blues

> …it is the unique make-up … of the cross-reactive memory CD8 + T cells … that determines the response to heterologous infection…

The diversity of the mammalian immune system is one of its most powerful attributes. Activation of cell-mediated immunity by foreign antigens induces a diverse range of T cells, each clone bearing a T-cell receptor (TCR) with unique specificity. But sometimes, for reasons that have so far been unclear, the T-cell response to viral infection is restricted, consisting of only a limited range of T-cell clones. New work by Liisa Selin, Raymond Welsh, Markus Cornberg and colleagues reveals that the unique composition of an individual's memory T-cell pool may lie at the heart of this dysfunctional immune response.

The authors suspected that activation of cross-reactive memory T cells might be implicated in generating a restricted T-cell response and they used a mouse model of virus infection to investigate this further. They first showed that epitopes from lymphocytic choriomeningitis virus (LCMV $NP_{205–212}$) and Pichinde virus (PV $NP_{205–212}$) are cross-reactive: NP_{205}-specific $CD8^+$ T cells that are activated by primary infection with LCMV or PV produced IFN-γ in response to the heterologous NP_{205} peptide. Sequence analysis of the Vβ portion of the TCR (a region of the TCR that varies among T cells) showed that NP_{205}-specific $CD8^+$ T cells activated during acute LCMV or PV infection have a broad and diverse TCR repertoire. TCRs from NP_{205}-specific $CD8^+$ T cells obtained from LCMV-infected mice revealed hundreds of different TCR Vβ sequences or clonotypes. Once acute infection with LCMV or PV resolves, a pool of memory $CD8^+$ T cells is established, including the cross-reactive NP_{205}-specific $CD8^+$ T cells. The authors went on to show that these cross-reactive T cells can have a profound influence on the memory T-cell repertoire. In PV-immune mice that are infected with LCMV, proliferation of cross-reactive PV NP_{205}-specific memory $CD8^+$ T cells results in a dominant NP_{205} response. But in contrast to the diverse $CD8^+$ T-cell population induced by NP_{205} in acute infection, the TCR repertoire of NP_{205}-specific $CD8^+$ T cells after heterologous LCMV challenge is limited and dominated by specific Vβ clonotypes. These results indicate that only a small subset of the cross-reactive NP_{205}-specific $CD8^+$ T cells proliferate after heterologous infection. This oligoclonal TCR repertoire can favour the generation of viral escape mutants as shown by the isolation of an LCMV NP_{205}-variant virus from PV-immune mice 8 months after LCMV challenge.

Using adoptive transfer experiments, Cornberg *et al.* revealed

BACTERIAL PHYSIOLOGY

Precious metal

Riboswitches are conformational switches in complex folded RNA domains that are induced by binding to specific small molecules, and which lead to a switch in gene-regulatory function. In a recent issue of *Cell*, Eduardo Groisman and his team at the Howard Hughes Medical Institute in St Louis, USA, report on the first example of a riboswitch that senses, and responds to, a metal ion.

Magnesium (Mg^{2+}) ions are abundant in biological systems and are required for a wide variety of cellular processes and structures. Cells maintain cytoplasmic Mg^{2+} levels within a narrow range, but little is known about the mechanisms through which cells sense and maintain these Mg^{2+} levels. *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) contains the Mg^{2+}-responding PhoP–PhoQ two-component regulatory system and several Mg^{2+} transporters. One of these transporters is MgtA, which mediates Mg^{2+} influx. The *mgtA* gene is regulated by the transcriptional activator PhoP in response to extracytoplasmic Mg^{2+} sensed by the sensor kinase PhoQ. In addition to the PhoP–PhoQ system, it was suspected that *mgtA* is regulated by another, Mg^{2+}-sensing, system, as *mgtA* transcription still responded to Mg^{2+} in an *S. typhimurium* strain lacking PhoQ and harbouring a Mg^{2+}-independent PhoP mutant. This Mg^{2+}-sensing mechanism is what Cromie *et al.* set out to investigate.

> …the first example of a riboswitch that senses, and responds to, a metal ion.

First, they used RT-PCR to examine the levels of *mgtA* mRNA in wild-type *S. typhimurium* strains that had been grown in the presence of different Mg^{2+} concentrations. They found that the *mgtA* transcript reaches the coding region only when *S. typhimurium* experiences very low Mg^{2+} concentrations, despite *mgtA* transcription being initiated at Mg^{2+}

that it is the unique make-up (or the private specificity) of the cross-reactive memory CD8$^+$ T cells of individual mice that determines the response to heterologous infection, although so far the mechanisms that favour the growth of some of these cells over others remain unknown.

The cross-reactive profiles of private T-cell repertoires might explain why some patients mount oligoclonal T-cell responses to HIV or hepatitis C virus infection and seem to be prone to the development of viral escape mutants. Finally, these results have important implications for vaccine design, as, in certain individuals, immunizing peptides could generate a pool of cross-reactive memory CD8$^+$ T cells that respond to subsequent heterologous virus challenge with a restricted T-cell repertoire.

Shannon Amoils

ORIGINAL RESEARCH PAPER Cornberg, M. *et al*. Narrowed TCR repertoire and viral escape as a consequence of heterologous immunity. *J. Clin. Invest.* 13 April 2006 (doi:10.1172/JCI27804)

concentrations 100-fold higher. Further experiments showed that the 5′-UTR region of *mgtA* is necessary, and sufficient, to respond to Mg^{2+}.

In a next step, Groisman and colleagues used the Mfold program to predict the secondary structure that might be adopted by the *mgtA* 5′-UTR. They identified two potential mutually exclusive structures: one including two stem-loop structures, A plus B, and an alternative stem-loop structure, C. Using *S. typhimurium* mutant strains where *mgtA* transcription initiated at different positions, it was found that stem loop B might be the structure formed when Mg^{2+} levels are high, and might be responsible for transcription stopping before the *mgtA* coding region. On the other hand, formation of stem loop C, the alternative to stem loops A plus B, might be favoured when Mg^{2+} levels are low.

Synthesizing the full-length *mgtA* 5′-UTR, and treating the RNA with RNases and chemicals to probe its structure after incubation in the presence of different Mg^{2+} concentrations, Cromie *et al.* showed that Mg^{2+} can modify the structure of the *mgtA* 5′-UTR. Stem loop A was found to be crucial for Mg^{2+} sensing, and for the Mg^{2+}-promoted changes taking place in stem loops B and C. Finally, using an *in vitro* transcription system, they demonstrated that the *mgtA* 5′-UTR responds to Mg^{2+} by affecting the ability of RNA polymerase to stop transcription.

So, the expression of MgtA is controlled by Mg^{2+} in two steps — the initiation of *mgtA* transcription by PhoP, which is controlled by PhoQ in response to extracytoplasmic Mg^{2+}, and the early stopping of *mgtA* transcription by its 5′-UTR riboswitch, which responds to cytoplasmic Mg^{2+} — in contrast to most genes that are controlled by riboswitches, which are typically only regulated by their respective riboswitches. Although this is the first evidence of a cation-responsive riboswitch, it is likely that additional ion-regulated riboswitches exist.

Annie Tremp

ORIGINAL RESEARCH PAPER Cromie, M. J., Shi, Y., Latifi, T. & Groisman, E. A. An RNA sensor for intracellular Mg^{2+}. *Cell* **125**, 71–84 (2006)

IN BRIEF

BACTERIAL PHYSIOLOGY

Definition of the bacterial *N*-glycosylation site consensus sequence

Kowarik, M. *et al. EMBO J.* 13 April 2006 (doi:10.1038/sj.emboj.7601087)

In eukaryotes, the consensus sequence for *N*-linked glycosylation is N–X–S/T (where X represents any amino acid except proline). In this work, Kowarik *et al.* investigated the substrate requirements for bacterial *N*-glycosylation *in vivo* and found the *Campylobacter jejuni* protein glycosylation machinery recognizes the consensus sequence D/E–Y–N–X–S/T (where X and Y can be any amino acid except proline). In addition to having an extended consensus sequence, Kowarik *et al.* demonstrated that the bacterial glycosylation system also shows increased stringency and specificity compared with eukaryotes. These results have implications for the biotechnological production of glycoproteins in bacteria.

VIROLOGY

Role of the α/β interferon response in the acquisition of susceptibility to poliovirus by kidney cells in culture

Yoshikawa, T. *et al. J. Virol.* **80**, 4313–4325 (2006)

In vivo, poliovirus, the causative agent of poliomyelitis, has a strict neurotropism, replicating at only a few sites including the brain and spinal cord. Fortunately for researchers working on poliovirus, the pioneers of early laboratory techniques for poliovirus cultivation, including John Enders and Renato Dulbecco, discovered that this neurotropism was not strictly observed *in vitro* and that cell lines of monolayer cultures derived from almost any primate tissue are susceptible to poliovirus infection. However, the molecular basis for the acquisition of poliovirus susceptibility by cultured cells has always been unknown. Now, reporting in the *Journal of Virology*, Yoshikawa *et al.* have shown that changes in the antiviral activity of the interferon response are likely to be the most important factors determining the acquisition of poliovirus susceptibility.

ANTI-INFECTIVES

Co-expression of virulence and fosfomycin susceptibility in *Listeria*: molecular basis of an antimicrobial *in vitro–in vivo* paradox

Scortti, M. *et al. Nature Med.* 23 April 2006 (doi:10.1038/nm1396)

The so-called '*in vitro–in vivo*' paradox for antibiotics refers to discrepancies between the resistance to a particular compound that is observed using *in vitro* susceptibility tests and the treatment outcomes that are observed for this compound in the clinic; that is, antibiotics that have poor efficacy *in vitro* can be successfully used to treat infections *in vivo*. In this Brief Communication in *Nature Medicine*, the authors present a molecular mechanism that could explain the paradox in fosfomycin treatment of *Listeria monocytogenes* infection. In *in vitro* susceptibility tests *L. monocytogenes* is resistant to fosfomycin, yet Scortti *et al.* demonstrate that in mice fosfomycin does have anti-listerial activity. They found that *L. monocytogenes* uptake of fosfomycin is dependent on the Hpt transporter and its regulator PrfA. PrfA is only weakly expressed under *in vitro* conditions but is highly induced *in vivo*, therefore explaining why *L. monocytogenes* is resistant to fosfomycin *in vitro*, but not *in vivo*.

PARASITOLOGY

Who's HOSTing who?

How the obligate intracellular pathogen *Toxoplasma gondii* acquires the nutrients that are necessary for its survival and growth has long been an open question. Reporting in *Cell*, Isabelle Coppens and colleagues now describe a novel mechanism by which *T. gondii* gains access to key molecules: sequestration of organelles of the host-cell endocytic pathway.

Infection of host cells with *T. gondii* involves internalization of the parasite into a membrane-bound compartment known as the parasitophorous vacuole, which — in contrast to most other pathogen-containing compartments — does not fuse with host-cell endocytic organelles. *T. gondii* therefore avoids the degradation that would result from sequential fusion with compartments of the endocytic pathway; however, in this way, it is not exposed to the proteins and lipids, such as cholesterol (for which *T. gondii* is auxotrophic), that enter the host cell through this pathway. Coppens and colleagues, however, had previously observed that cholesterol is somehow transferred from host endocytic organelles to the parasitophorous vacuole, so they set out to examine the localization of host endocytic organelles in *T. gondii*-infected cells, using fluorescence microscopy and transmission electron microscopy.

After infection with *T. gondii*, host endolysosomes were found to accumulate around the parasitophorous vacuole, which was also shown to be surrounded by networks of host microtubules. This cytoskeletal reorganization was found to result in the formation of invaginations — referred to as host organelle-sequestering tubulo-structures (HOST) — of the membrane of the parasitophorous vacuole. The authors propose that the tubular structure of HOST allows them to function as conduits for the delivery of endolysosomes to the parasitophorous vacuole. Furthermore, HOST were found to be coated with parasite proteins, including the granule protein GRA7, which can constrict the HOST conduits, thereby preventing exit of the host organelles and sequestering them within the parasitophorous vacuole. The authors postulate that the contents of these organelles would then undergo hydrolysis, and cholesterol and other important low-molecular-weight molecules would be released across the membrane of the intact organelles and absorbed by the parasite.

Not only is this an unexpected finding of a novel process of nutrient acquisition by a parasite, but it is also a unique mechanism for the unidirectional transport and sequestration of host-cell organelles.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Coppens, I. *et al. Toxoplasma gondii* sequesters lysosomes from mammalian hosts in the vacuolar space. *Cell* **125**, 261–274 (2006)
FURTHER READING
Gruenberg, J. & and Gisou van der Goot, F. Toxoplasma: Guess who's coming to dinner. *Cell* **125**, 226–228 (2006)
WEB SITE
Isabelle Coppens's homepage: http://faculty.jhsph.edu/?F=Isabelle&L=Coppens

ENVIRONMENTAL MICROBIOLOGY

A global unculture…

Two reports recently published in *Nature* shed new light on two processes only recently identified as being major contributors to global carbon and nitrogen cycling. Both processes — the anaerobic oxidation of ammonium and methane — were thought to be non-existent in nature but are, in fact, catalysed by unrelated microorganisms that have yet to be grown in pure culture.

The obligate anaerobic ammonium oxidation (anammox) reaction, which uses nitrite as the primary electron acceptor, is accountable for up to 50% of oceanic nitrogen loss. Although identified in 1999, the planctomycete-like bacteria that catalyse this process have proven difficult to study owing to their extremely slow growth. Now, Strous *et al.* have applied the advantages of environmental genomics to gain insight into the unusual biology of these important microorganisms. Anammox bacteria have a unique prokaryotic organelle (the 'anammoxosome') surrounded by ladderane lipids that contain hydrazine oxidoreductase,

The photograph shows Marc Strous extracting a mud sample from the Twentekanaal, a canal in the Netherlands. These anoxic sediments were used as the inoculum for the enrichment of an anaerobic methane-oxidizing microbial consortium. Photograph courtesy of Radboud University Nijmegen.

FUNGAL PATHOGENESIS

Global control

Researchers have identified the regulator of the phase transition from the non-pathogenic hyphal form to the pathogenic yeast form in dimorphic fungal pathogens, according to a paper published recently in *Science*.

Six dimorphic ascomycetes including *Blastomyces dermatitidis* and *Histoplasma capsulatum* are typically found in the environment but can cause disease in humans if spores are inhaled. Temperature is the key environmental cue for fungal dimorphic switching, with the transition occurring with a shift from 25°C to 37°C, but the key outstanding question in the field has been what regulates this phase transition. In this work, Julie Nemecek, Marcel Wüthrich and Bruce Klein set out to identify the master regulator that controls the switch from the hyphal to the yeast form.

Previous work in the Klein laboratory had shown that *Agrobacterium tumefaciens* DNA could be transformed randomly into the *B. dermatitidis* genome and so Nemecek *et al.* devised an insertional mutagenesis assay in which *A. tumefaciens* DNA was transformed into a *B. dermatitidis* reporter strain. Blue/white selection identified seven transformants and the transformant chosen for further analysis had an 86% reduction in transcription of the yeast-phase-specific reporter gene (*BAD1*) compared with the parental strain. Further analysis of the mutant

an enzyme that combines nitrite and ammonia in a one-to-one mechanism. Using an anoxic laboratory bioreactor that contained a complex microbial community dominated by the anammox bacterium *Kuenenia stuttgartiensis*, the authors sequenced over 1 gigabase of extracted DNA. These sequences data allowed the researchers to assemble the genome of *K. stuttgartiensis,* one of the first complete genome sequences of an organism not available in pure culture. Analysis of the genome revealed the presence of over 200 genes directly involved in anammox catabolism and respiration, as well as candidate genes responsible for ladderane biosynthesis and biological hydrazine metabolism. This large number of genes is also indicative of an unexpected degree of metabolic versatility in the microorganism.

Environmental sequence analysis was also used to analyse the genomes of a microbial consortium that coupled the direct oxidation of methane to denitrification of nitrate in the absence of oxygen. In this separate study, Marc Strous and colleagues enriched for microbial life derived from the anoxic sediments of a freshwater canal over a 16-month period. Analysis of the culture revealed the presence of two different types of microorganism, a bacterium belonging to a novel phylum without any documented cultured species, and an archaeon distantly related to marine methanotrophic archaea. Interestingly, similar sequences were also identified in freshwater ecosystems from different global locations, strongly suggesting that the process of microbial anaerobic methane oxidation coupled to denitrification has worldwide ecological significance.

The contribution of microorganisms to global biogeochemical cycles is well established; however, important aspects pertaining to their biology have remained ill-defined or overlooked. These two studies represent significant advances in addressing these gaps and clearly show the power of genomics in elucidating the biology of environmentally important, yet 'unculturable', microorganisms.

David O'Connell

ORIGINAL RESEARCH PAPERS Strous, M. *et al.* Deciphering the evolution and metabolism of an anammox bacteria from a community genome. *Nature* **440**, 790–794 (2006) | Raghoebarsing, A. A. *et al.* A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440**, 918–921 (2006)

phenotype revealed a pleiotropic set of defects, including the inability to undergo the switch from the hyphal to the yeast form.

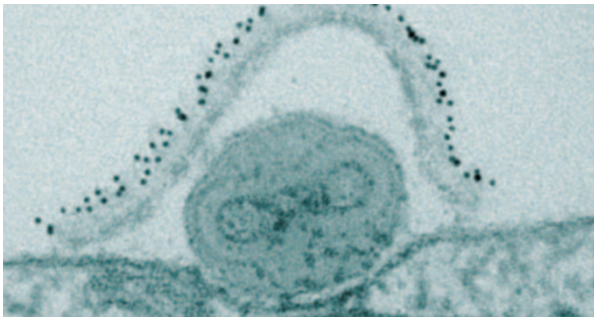
The authors went on to map the site of insertion in the *B. dermatitidis* genome and identified the disrupted gene as an open-reading frame (ORF) encoding a 1274-residue protein. Sequence analysis revealed that this ORF encodes a hybrid histidine kinase with homology to the *Saccharomyces cerevisiae* histidine kinase *SLN1*. Knocking out the ORF by allelic replacement induced the same pleiomorphic defects as observed in the original transformant and so the authors named the gene *DRK1*, for dimorphism-regulating histidine kinase.

In addition to having a homologue in the *S. cerevisiae* genome, *DRK1* is also conserved in the other dimorphic fungal pathogens for which extensive sequence information is available, including *H. capsulatum* and *Coccidioides posadasii*. RNA interference was used to silence *DRK1* expression in *B. dermatitidis* and *H. capsulatum* and it was found that in *H. capsulatum*, as in *B. dermatitidis*, *DRK1* is involved in dimorphic switching and virulence-gene expression. Finally, *in vivo* analysis showed that the pathogenicity of *DRK1*-silenced strains of both *B. dermatitidis* and *H. capsulatum* was reduced in murine models of infection.

This study represents a milestone in research for those interested in dimorphic fungal pathogens as it not only identifies *DRK1* as a key global regulator that controls the hyphal-to-yeast transition, virulence-gene expression and pathogenicity but also finally confirms that the transition to the yeast form is a requirement for pathogenicity.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Nemecek, J. C., Wüthrich, M. & Klein, B. S. Global control of dimorphism and virulence in fungi. *Science* **312**, 583–588 (2006)



Immuno-electron microscopy showing non-fusogenic disruption of the EEV outer membrane, which exposes the IMV particle to the cell surface. Image reproduced with permission from Law *et al.* © (2006) National Academy of Sciences, USA.

VIROLOGY

A novel state of undress

Enveloped viruses penetrate target cells by fusing their single lipid membrane with the target cell membrane and releasing the infective naked viral core. But some virus forms such as the extracellular enveloped virus (EEV) of *Vaccinia virus* have two lipid membranes, and therefore cell entry requires the removal of both lipid barriers. New work from Geoffrey Smith's laboratory at Imperial College London has uncovered a unique way in which EEV sheds its outer lipid membrane, providing novel insights into virus entry and also a new target for antiviral therapy.

The authors studied the binding and entry of EEV to cells by immuno-electron microscopy and observed that the outer EEV membrane ruptures at the site of cell contact. This disruption, however, takes place in the absence of membrane fusion and the shed EEV outer membrane remains draped over the underlying single-enveloped virion, which is called an intracellular mature virus (IMV). The single membrane of IMV subsequently fuses with the target cell membrane and the virus core enters the cell.

Because disruption of the EEV outer membrane takes place only on contact with a cell, and not a synthetic substrate, the authors proposed that outer membrane dissolution required the interaction between ligands on the virus and the target cell surface. A series of experiments identified the virus glycoproteins and, and cellular surface polyanions, known as glycosaminoglycans, as the required ligands. This is the first example of the removal of a virus membrane without fusion, which Smith and colleagues term ligand-dependent non-fusogenic viral membrane dissolution.

The authors went on to show that a soluble polyanion such as heparin can be used to rupture the EEV outer envelope *in vitro*, exposing the IMV and allowing neutralization by anti-IMV monoclonal antibodies. The combined administration of polyanions and anti-IMV antibodies protected mice against disease with *Vaccinia virus*, demonstrating the therapeutic potential of this strategy.

The EEV virion is the form of *Vaccinia virus* that spreads within the host and therefore an increased understanding of its entry mechanism and the identification of a strategy to target EEV entry are important advances in this field.

Shannon Amoils

ORIGINAL RESEARCH PAPER Law, M. *et al.* Ligand-induced and nonfusogenic dissolution of a viral membrane. *Proc. Natl Acad. Sci. USA* **11**, 5989–5994 (2006)