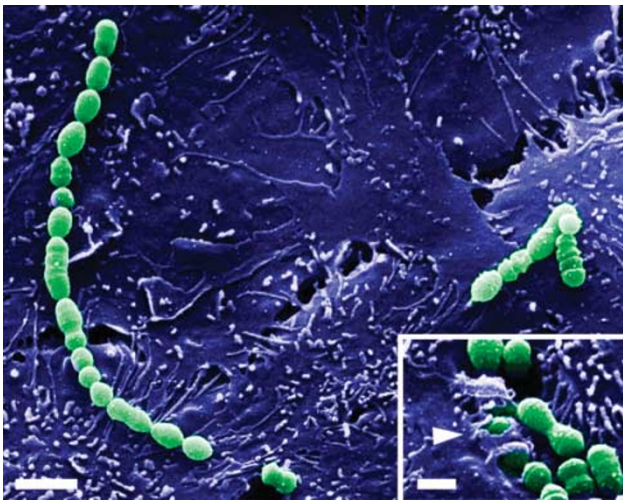
BACTERIAL PATHOGENESIS

Colonization control



Adhesion and internalization of *Streptococcus pyogenes* on an epithelial cell layer as visualized by scanning electron microscopy. The scale bar represents 2 μm. Bacteria being internalized are shown at higher magnification in the insert (scale bar 1μm). Photo courtesy of Matthias Mörgelin, Lund University, Sweden.

For many bacterial pathogens, adhesion to host cells and subsequent internalization are the two initial steps in the colonization process. For *Streptococcus pyogenes*, binding to fibronection via the cell-wall-attached fibronectin-binding receptor, protein F1, is a key molecular interaction that mediates these steps. Now, a report published in the May issue of *Microbiology* sheds new light on the regulation of this crucial interaction by revealing a role for the secreted bacterial protease, SpeB.

SpeB, which is secreted in large amounts by most *S. pyogenes* strains, has broad proteolytic activity against a number of different human proteins, as well as several *S. pyogenes* proteins that are located on the bacterial cell surface. Previous studies have also shown that SpeB contributes to bacterial internalization, but the mechanisms by which the protease influences this process were unclear. Patrik Nyberg and colleagues set out to clarify the role of SpeB in streptococcal entry into host cells, focusing on the requirement of fibronectin. Initially, the authors were able to demonstrate that growing *S. pyogenes* in a medium that promoted SpeB expression reduced the ability of the bacteria to bind to fibronectin. They were further able to show that this reduction in fibronectin-binding activity was due to the proteolysis of protein F1 by SpeB. As bacterial internalization is mediated by protein F1, the authors were also able to demonstrate that the removal of protein F1 from the bacterial cell surface by SpeB resulted in a significant decrease in adhesion and internalization. Unlike other surface proteins of *S. pyogenes* that are protected from proteolytic degradation by interacting with their ligands, protein F1 was readily cleaved by SpeB, even when complexed with fibronectin.

These data unambiguously show the sensitivity of the fibronectin-binding activity of *S. pyogenes* to the

CELLULAR MICROBIOLOGY

Recruitment drive

Legionella pneumophila is an intracellular pathogen that can avoid delivery to lysosomes — and certain death — after uptake by a host cell, by modifying the vacuole that it is constrained within to provide a safe place for bacterial replication. Research published in the *Journal of Experimental Medicine* reveals that *L. pneumophila* recruits the host trafficking proteins Rab1 and Sec22b to aid in the maturation of the vacuole into a replicative organelle.

After entering the host cell (usually a macrophage) *L. pneumophila* is confined in a *Legionella*-containing vacuole (LCV), which later matures into a replicative organelle. LCV maturation depends on the expression of the Dot/Icm bacterial secretion system, which injects bacterial proteins into the host cell cytosol. Microscopy and bacterial genetics have revealed that vesicles that exit the endoplasmic reticulum (ER), and are destined for transport to the Golgi, are redirected to fuse with the LCV. Subsequent remodelling of the LCV results in a protected niche — the replicative organelle — in which the bacteria multiply. Mechanisms that mediate the LCV maturation process, including the transport pathways that the bacterium affects, were investigated in this study.

Rab proteins function to transport vesicles between the ER and the Golgi, so Kagan *et al.* used immunofluorescence microscopy to investigate whether Rab proteins were recruited to the LCV. Of the three Rab proteins (Rab1, 2 and 6) that function in vesicular transport between the ER and the Golgi only Rab1 was localized to the LCV. This process depended on functioning of the Dot/Icm secretion system, but not on the exit of early secretory vesicles from the ER, so bacterial factors injected into the host cell presumably recruit Rab1. Since treatment of host cells to prevent vesicular transport did not prevent Rab1 recruitment to the LCV, Rab1 recruitment is independent of ER vesicle-mediated LCV-remodelling. Abrogation of cellular Rab1 function reduced the intracellular replication of *L. pneumophila* so Rab1 clearly has a role in conversion of the LCV into a competent replication organelle.

Sec22b, a SNARE protein that participates in vesicular fusion and is recruited by Rab proteins, was also recruited to the LCV, but again only if the Dot/Icm system was functional. In contrast with Rab1 recruitment, Sec22b recruitment required early ER vesicular transport, so it seems that Sec22b is recruited after Rab1. Kagan *et al.* disrupted Rab1 function, and used immunogold microscopy to visualize Sec22b, to show that Rab1 recruitment promotes the transport to, and fusion of, ER-derived vesicles that contain Sec22b with the LCV.

Dissecting the LCV-maturation process further to delineate the recruitment and vesicular fusion mechanisms will shed light not only on *Legionella* pathogenesis, but on the basic cell biology of phagosome maturation. This study clearly demonstrates the utility of bacterial model systems for cell biology research.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Kagan, J. C. *et al.* *Legionella* subvert the functions of Rab1 and Sec22b to create a replicative organelle. *J. Exp. Med.* **199**, 1201–1211 (2004)

WEB SITE

Craig Roy's laboratory:
http://info.med.yale.edu/micropath/fac_roy.html

proteolytic action of SpeB with the resulting effects on bacterial internalization. This function for SpeB could represent a mechanism for the proteolytic regulation of *S. pyogenes* entry into host cells. Indeed, as SpeB expression and secretion are thought to be induced when the bacteria are in an environment in which nutrients are limited, the authors speculate that proteolysis of protein F1 and the subsequent inhibition of adhesion and internalization, could promote bacterial dissemination by allowing the movement of *S. pyogenes* to new and more fertile locations.

David O'Connell

References and links

ORIGINAL RESEARCH PAPER Nyberg, P. *et al.* SpeB modulates fibronectin-dependent internalization of *Streptococcus pyogenes* by efficient proteolysis of cell-wall-anchored protein F1. *Microbiology* **150**, 1559–1569 (2004)

WEB SITE

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VIRAL PATHOGENESIS

Micro modulators

A new report in *Science* has revealed another weapon that viruses can use to control host gene expression: microRNAs.

MicroRNAs (miRNAs) are endogenously expressed, small (~22-nucleotide) RNAs that are known to regulate gene expression in plants and animals by targeting specific mRNAs for degradation or translational repression. Although the first miRNA was identified in *Caenorhabditis elegans* in 1993, the ubiquitous nature of miRNAs was not discovered until 2000. Since then, rapid progress has been made — miRNAs have been identified in all animal and plant genomes analysed to date and miRNAs are now known to be one of the most abundant gene regulatory molecules in animals.

Pfeffer *et al.* looked for evidence of the presence of miRNAs in Epstein–Barr virus (EBV), a γ-herpesvirus that is ubiquitous in human populations. EBV infects a variety of cell types, including epithelial cells and B cells, where it establishes a latent infection. EBV infection is associated with ~1% of all cancers worldwide, including Burkitt's lymphoma (BL) and nasopharyngeal carcinoma.

Pfeffer *et al.* cloned all the small RNAs from a BL cell line. Sequence analysis of the cloned EBV RNA indicated that the structural features were typical of those of miRNAs. Five EBV miRNAs were identified in total and are located in two clusters in the EBV genome. Three are located within the *BHFR1* coding region and have been named miR-BHRF1-1, -2 and -3, and the other two are located in the intronic region of the *BART* gene and have been named miR-BART1 and -2.

Latent infection with EBV occurs in distinct stages, during which different viral latent genes are expressed. To establish whether the EBV miRNAs were expressed during a particular stage, Pfeffer *et al.* screened a variety of cell lines at different stages of latent infection. miR-BART1 and -2 were detected at all stages of latency, consistent with the fact the *BART* is expressed throughout latent infection. By contrast, the expression of the miR-BHRF1 miRNAs was found to be stage-dependent. Interestingly, miR-BHRF1 was detected in one of two cell lines in latent stage I, which were previously thought to express only one EBV latent protein and EBERs (small EBV-encoded RNAs); Pfeffer *et al.* suggest that a new latent stage might need to be created to distinguish between cells that express BHFR1 miRNAs and those that do not.

In silico analysis to identify putative host cell targets for these miRNAs identified many possible targets, including regulators of cell proliferation and apoptosis. Of the putative targets identified, more than half are also targeted by miRNAs encoded by the host cell. Experimental evidence was also obtained to indicate that at least one of the EBV miRNAs can regulate EBV gene expression.

As viruses have perfected their control over host cells during millions of years of co-evolution, it is perhaps unsurprising that yet another method that viruses can use to manipulate host gene expression has been uncovered. Using non-coding RNAs to regulate gene expression is not only convenient but also non-immunogenic and could therefore also contribute to viral immune evasion. Pfeffer *et al.* anticipate that miRNAs will be identified in other γ-herpesviruses and other viruses with large DNA genomes.

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER Pfeffer, S. *et al.* Identification of virus-encoded microRNAs. *Science* **304**, 734–736 (2004)

FURTHER READING Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism and function. *Cell* **116**, 281–297 (2004)