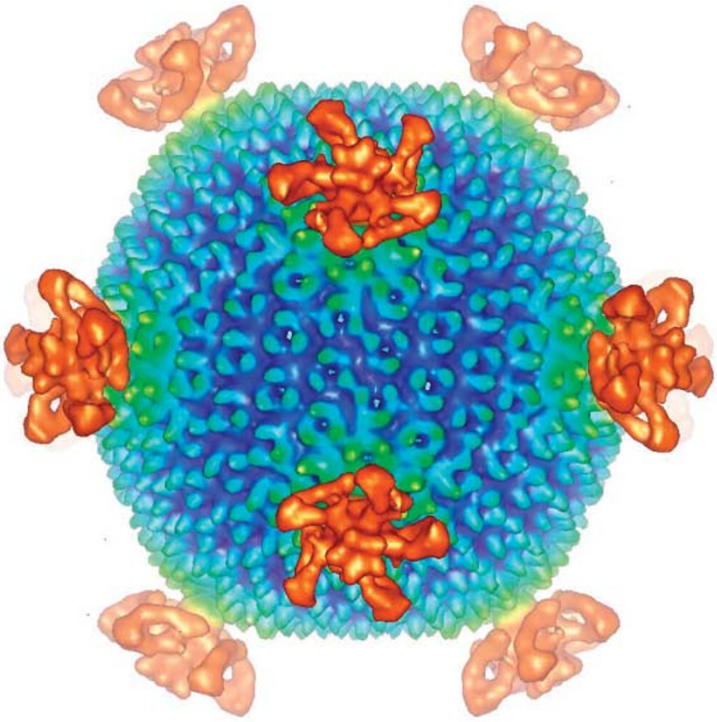
VIRUS STRUCTURE

Founding member



CryoTEM and image reconstruction of STIV. Turrets extend 13 nm above the virus surface. Image courtesy of Mark Young, (Montana State University-Bozeman, USA).

The structure of a virus that infects a hyperthermophilic archaeon has just been published in the *Proceedings of the National Academy of Sciences* (USA). The major coat protein in the capsid has striking similarities to both animal and bacterial viruses — raising the exciting prospect that some viruses might have a common ancestor from more than 3 billion years ago.

Of more than 5,000 viruses that have been characterized, only a tiny fraction are parasites of archaeal species. Hyperthermophilic archaea inhabit hostile niches such as boiling hot springs, and evolutionary analyses indicate that this group is one of the most ancient lineages in the tree of life. Virus evolution has not been as easy to address, owing to the lack of homology between viral genes. Now, attention has focused on structural similarities between viral proteins.

Viruses that replicate in archaeal species have been isolated in the past, including four viruses that replicate in *Sulfolobus* species, but each virus is unrelated to other viruses using genome sequence comparisons. Four new families have been created for these archaeal viruses: Fuselloviridae (spindle-shaped), Rudiviridae (rod-shaped), Lipothrixviridae (filamentous) and Guttaviridae (teardrop).

The virus isolated in this study replicates in a close relative of *Sulfolobus solfataricus*, but turned out to be different to the other viruses previously isolated from *Sulfolobus* species. The double-stranded DNA genome contained only 3 open reading frames (ORFs) that had any similarity to previously sequenced genes. Using cryoTEM (transmission electron microscopy) to solve the structure of the virus particle to 27-Å resolution, Rice and co-workers found surprising structural features. The capsid is icosahedral with unusual turret-like protruberances, so the authors have named this virus STIV — *Sulfolobus* turreted icosahedral virus.

Importantly, the STIV capsid has a similar organization to the capsids of the human adenovirus, bacteriophage PRD1 and an algal virus PBCV-1. Moreover, docking the crystal structures of the major capsid proteins of human adenovirus and PRD1 onto the STIV major capsid protein revealed that all three proteins have a

BACTERIAL PHYSIOLOGY

Positive secretion

A fundamental challenge confronted by all organisms concerns the successful secretion of biologically active proteins across cellular membranes. Now, a report just published in *Science* details the discovery of one mechanism used by Gram-positive bacteria to export proteins crucial to their survival and proliferation.

Unlike the periplasmic space of Gram-negative bacteria or the endoplasmic reticulum of eukaryotic cells, Gram-positive bacteria lack a specialized compartment external to the cell membrane that allows folding of secreted proteins. To address the question of how these bacteria export proteins, Jason Rosch and Michael Caparon focused on the secretion mechanisms of the important human pathogen, *Streptococcus pyogenes*, a Gram-positive microorganism that secretes more than 40 proteins as part of its pathogenic strategy. Genome analysis had revealed that the streptococci contain only the general secretory (Sec) pathway for protein export across the cytoplasmic membrane. What remained unclear, however, was an understanding of how the Sec pathway functioned and was organized in this microorganism. Using both immunogold electron microscopy and fluorescence microscopy to probe for a secreted streptococcal virulence factor (SpeB), the authors were able to demonstrate that secretion occurred at a single microdomain in the cellular membrane. The authors were further able to show the same targeted localization with a non-streptococcal protein (PhoZ), indicating that this secretion mechanism is a general phenomenon and is not restricted to SpeB. Together, these findings clearly establish that protein secretion in *S. pyogenes* occurs at a distinct microdomain of the cytoplasmic membrane dedicated to protein export, a domain that the authors have named the Exportal. As the asymmetric secretion of molecules is essential to many processes in bacteria, Rosch and Caparon further investigated the basis for this targeted localization of secreted proteins. Again, using immunogold electron microscopy, the authors were able to show that secreted proteins and the Sec translocation machinery co-localized to the Exportal, a finding that is consistent with a model in which the targeting of the secretion proteins results from the ability of the microdomain to accumulate high concentrations of the Sec translocons.

These data represent a new mechanism for asymmetric protein secretion using the Sec pathway, and constitute a significant advance in our knowledge of streptococcal protein secretion. A more intriguing possibility is that this secretion process might also represent a paradigm for secretion common to all Gram-positive bacteria. Future work will be needed to address this possibility as well as the contributions of the Exportal to host–pathogen interactions.

David O'Connell

References and links

ORIGINAL RESEARCH PAPER Rosch, J. & Caparon, M. A microdomain for protein secretion in Gram-positive bacteria. *Science* **304**, 1513–1515 (2004)

WEB SITE

Michael Caparon's laboratory:
http://www.microbiology.wustl.edu/dept/fac/caparon.html

similar arrangement of structural features including β-sheets. Plus, in common with the animal and bacterial viruses, there might be a lipid envelope present, which must be confirmed biochemically.

The lack of ORFs that are conserved between viruses isolated from archaeal species has been puzzling because it might be expected that important genes encoding proteins that govern genome replication, for example, would be conserved. Perhaps parallel evolution in these ecologically isolated species generated their incredible diversity. Going back to basics and using shape as a defining characteristic, like traditional naturalists, has borne fruit for these researchers. By coupling genomics with structural biology, the lineages of the tree of life — even for viruses — might yet be defined.

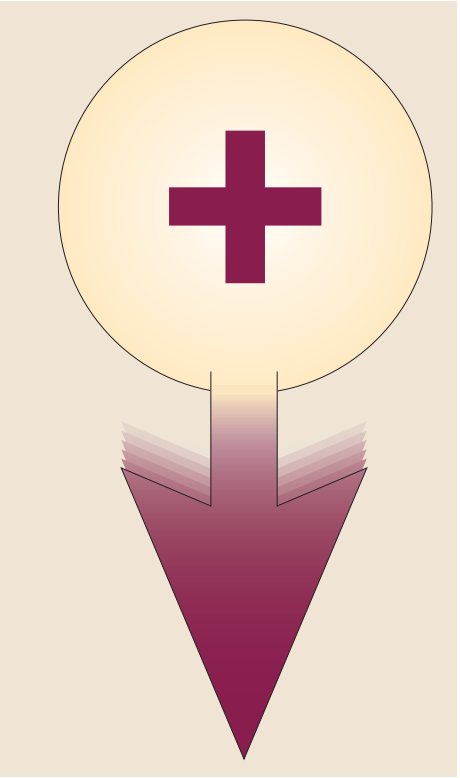
Susan Jones

References and links

ORIGINAL RESEARCH PAPER Rice, G. *et al.* The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proc. Natl Acad. Sci. USA* **101**, 7716–7720 (2004)

WEB SITE

Mark Young's laboratory:
http://www.homepage.montana.edu/~markyoung/default.htm



BACTERIAL PATHOGENICITY

Step by step

More than 50 years after the first bacterial type IV secretion system (T4SS) — the conjugation apparatus of the F-plasmid — was described, a new report in *Science* has defined the specific translocation pathway for a DNA substrate through a T4SS.

Bacteria use T4SSs for a variety of fundamental biological functions, including the exchange of genetic material with other bacteria and the translocation of oncogenic DNA and pathogenic effector proteins into eukaryotic host cells. Research on T4SSs has generated a large body of knowledge regarding the genes and proteins involved, the structure of the secretory apparatus and the effects of the translocated substrates on host cells. Now, Cascales and Christie have gone one step further and developed a method to determine the sequential pathway of DNA translocation through the archetypal *Agrobacterium tumefaciens* VirB/D4 T4SS.

Cascales and Christie developed a sensitive assay based on the chromatin immunoprecipitation (ChIP) assay, a technique that had previously been used to study specific protein–DNA binding in both eukaryotes and prokaryotes. Cascales and Christie call their variation transfer DNA immunoprecipitation (TrIP), and the assay has three stages — formaldehyde treatment of *A. tumefaciens* cells induces *in vivo* cross-linking between DNA and proteins, the proteins of interest are immunoprecipitated and the co-precipitation of DNA with the proteins of interest is then analysed using a PCR assay that can be quantitative if required.

The *A. tumefaciens* system comprises 11 VirB proteins (VirB1–VirB11) plus associated VirD proteins. The VirD2 relaxase cleaves the transfer DNA (T-DNA) and binds covalently to the single-stranded product, forming a VirD2–T-DNA transfer intermediate. Cascales and Christie analysed the sequential involvement of the VirB proteins in the translocation of the VirD2–T-DNA substrate using the quantitative TrIP assay and a series of mutants each lacking a single VirB protein.

The five VirB proteins that precipitated the most T-DNA (VirB2, -B6, -B8, -B9 and -B11) are classified by the authors as class I T4SS subunits, and it is proposed that they are distributed across the cell envelope and form the channel of the secretory apparatus. The remaining six VirB proteins were divided into two classes. For the class III subunits (VirB1 and -B3 and the accessory protein VirJ), there was no evidence of contact with the T-DNA. The class II subunits (VirB4, -B5, -B7 and -B10) interacted with low amounts of T-DNA, and evidence was presented that these subunits probably precipitated T-DNA indirectly through formaldehyde cross-linking to class I subunits.

The results of this sensitive, systematic analysis provide proof for the hypotheses of the contributions of individual subunits that had been generated from previous studies. Cascales and Christie conclude that 'TrIP should also prove highly useful for studies of many other fundamental processes…that involve the movement of DNA across biological membranes, including bacterial transformation and transduction…and viral infection cycles.'

Sheilagh Clarkson

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ORIGINAL RESEARCH PAPER Cascales, E. & Christie, P. J. Definition of a bacterial type IV secretion pathway for a DNA substrate. *Science* **304**, 1170–1173 (2004)

FURTHER READING Cascales, E. & Christie, P. J. The versatile type IV secretion systems. *Nature Rev. Microbiol.* **1**, 137–149 (2003)

WEB SITE

Peter Christie's laboratory:
http://mmg.uth.tmc.edu/webpages/faculty/pchristie.html