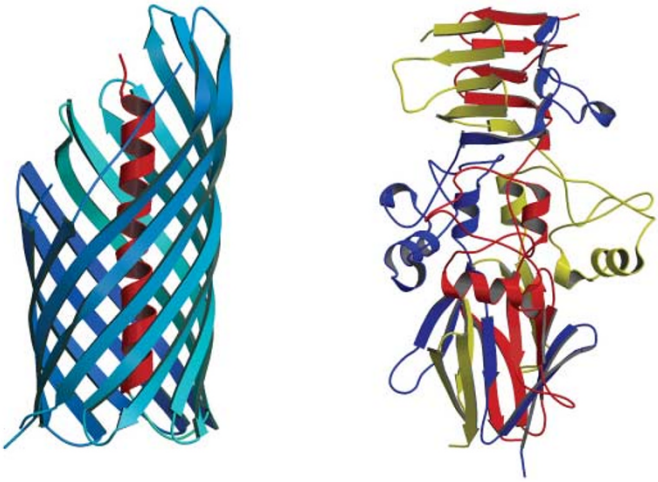
BACTERIAL SECRETION

Autotransporters — monomers and multimers



Ribbon diagrams of the translocator domain of the classic autotransporter protein NalP (left) and the primary binding domain of Hia (right), a member of the recently recognized trimeric autotransporter subfamily. The helix inserted into the pore of NalP (left) is shown in red. Each subunit of the Hia adhesin (right) is colour-coded differently. Image courtesy of C. Oomen, Birkbeck College, London, UK.

Protein secretion from Gram-negative bacteria involves transport across both the inner and outer membranes. Now, two high-resolution structures in *EMBO Journal* shed light on the mechanisms of secretion by the autotransporter system and the role of these secreted proteins in virulence.

Autotransporters consist of three domains: an N-terminal signal peptide, which directs export of the precursor protein across the inner membrane before being cleaved; a C-terminal translocator, or β, domain, which inserts into the outer membrane; and a passenger domain, which is surface localized and often released from the bacterium.

Piet Gros and colleagues have solved a high-resolution X-ray crystal structure of the translocator domain of NalP from *Neisseria meningitidis*. The structure reveals a 12-stranded β-barrel with a hydrophilic pore of 10 × 12.5 Å — a classical structure for outer-membrane proteins. But, unusually, the pore is filled with an N-terminal α-helix. Previous studies have proposed two distinct models for the protein-translocation process: transportation of the passenger domain through a pore formed by a single translocator domain; and transportation of the passenger domain through a pore formed by a multimer of translocator domains. The presence of hydrophobic residues on the exterior of the β-barrel seems inconsistent with the multimeric model; however, on the basis of the crystal structure the monomeric model would involve translocation of an unfolded protein. For these reasons, they propose an alternative mechanism that requires the Omp85 complex (which is required for the assembly of integral outer-membrane proteins).

In a separate study, Gabriel Waksman, Joseph St Geme and colleagues report the X-ray crystal structure of the HiaBD1 region of the passenger domain of the *Haemophilus influenzae* Hia protein — an adhesin that is primarily responsible for the binding of *H. influenzae* to host cell receptors. Unlike NalP, the passenger

FUNGAL PHYSIOLOGY

A spot of light training?

Clock genes, which are central to circadian regulation, have been found in all organisms. How clocks are synchronized with, or entrained to, a 24-h day, and adjusted to different seasonal daylengths is, however, still a puzzle. A new report published in *Current Biology* reveals that entrainment of the model fungus *Neurospora crassa* clock is more complex than previously thought. Rather than a simple transcriptional feedback regulatory loop, translation and protein stability also impinge on clock control.

Light, the most important environmental training signal, can switch clock genes on, and promote degradation of clock proteins. Multiple input proteins feed light signalling into the *N. crassa* clock, but entrainment is crucially dependent on the *frq* gene. *frq* is negatively regulated by FRQ protein abundance, but because *frq* is strongly induced by a pulse of light, it was previously thought that the fungal clock was light-driven, and that the effects of light could over-ride autorepression by the FRQ protein.

Here, Tan *et al.* show that during a 24-h day the *frq* gene is strongly induced at 'lights-on', and decreases after 'lights-off', so the clock does seem to be light-driven. What happens to the FRQ protein though, during daily cycles?

Using 24-h days with different photoperiods — short days/long nights or short nights/long days — FRQ abundance was upregulated after lights-on, but the timing of this was found to depend on the length of the night. Long nights resulted in a rapid upsurge in abundance of the FRQ protein after lights-on, whereas after short nights, abundance of the FRQ protein was upregulated after lights-on, but with a considerable delay. This indicates that transcription and translation at the *frq* locus have become dissociated by the length of the photoperiod. Plus, levels of the FRQ protein declined after lights-off at different rates, dependent on the photoperiod. Half-maximal levels were always reached at around midnight, whatever the photoperiod, within a 24-h day.

To attempt to reconcile this with the known response of spore formation (conidiation), which always occurs 7 h after lights-off in 50% light/dark cycles of varying lengths, conidiation was carried out under the same conditions. Using a 24-h day with varying day/night lengths, conidiation always occurred around midnight — when the FRQ protein was at half-maximal levels — independent of the photoperiod. It seems that FRQ abundance regulates conidiation.

Expression of the clock component protein FRQ integrates environmental history — depending on the photoperiod, the FRQ protein declines at different rates — whereas the *frq* gene is always upregulated in response to light. So the *N. crassa* clock is not, after all, solely driven by light, but fluctuates during a light/dark cycle like the clocks of higher organisms. Biochemistry and genetics will now be needed to tease out how FRQ degradation is modulated.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Tan, Y. *et al.* Entrainment dissociates transcription and translation of a circadian clock gene in *Neurospora*. *Curr. Biol.* **14**, 433–438 (2004)

FURTHER READING Liu, Y. Molecular mechanisms of entrainment in the *Neurospora* circadian clock. *J. Biol. Rhythms* **18**, 195–205 (2003)

WEB SITES

Till Roenneberg's laboratory: http://www.imp-muenchen.de/?chronobiology

Encyclopedia of Life Sciences: http://www.els.net Circadian rhythms in *Neurospora*

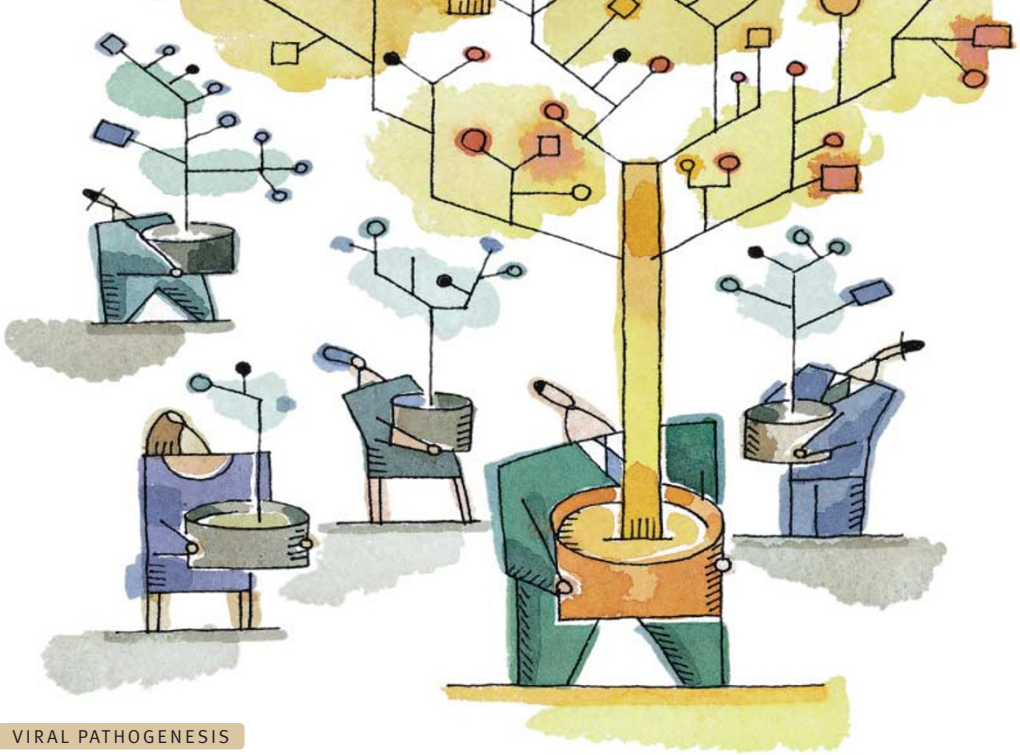
domain of Hia is not cleaved and remains attached to the translocator domain and, in contrast to the NalP structure, the HiaBD1 structure reveals a novel trimeric arrangement of individual HiaBD1 subunits, which has implications for the virulence of *H. influenzae* as it could enable multivalent interactions with the host cell. Sequence comparisons indicate that HiaBD1 is a member of a new subfamily of autotransporters in which both the passenger and translocator domains trimerize.

These studies provide clues as to the mechanisms and role of two autotransporters; however, further research is needed to determine not only if they do indeed represent two distinct classes of autotransporters, but also if the Omp85 complex is involved.

Jane Saunders

References and links

ORIGINAL RESEARCH PAPERS Oomen, C. J. *et al*. Structure of the translocator domain of a bacterial autotransporter. *EMBO J.* **23**, 1257–1266 (2004); Yeo, H.–J. *et al*. Structural basis for host recognition by the *Haemophilus influenzae* Hia autotransporter. *EMBO J.* **23**, 1245–1256 (2004)



VIRAL PATHOGENESIS

Strategic comparisons

Studies of viral pathogenesis in model hosts, such as rodents, are often extrapolated to humans. But are the pathogenic strategies of viruses in different cell types really comparable? A study just published in the *Journal of Virology* tackles this question using a global transcriptome approach.

Pseudorabies virus (PRV) and herpes simplex virus type 1 (HSV-1) are α-herpesviruses, which, despite low overall sequence identity, have conserved genome organization, virion structure and replication cycles. PRV infects pigs and HSV-1 infects humans — in both cases only mild symptoms occur. PRV cannot infect humans, and HSV-1 cannot infect pigs, but both viruses infect rodents, with lethal effect. Because the replication cycles in human, porcine and rodent cells are similar, and because viral strains that are attenuated in the natural host are also attenuated in rodents, pathogenic mechanisms could be conserved.

Ray and Enquist used transcriptome studies of PRV and HSV-1 infection of rodent cells to ask a simple question. Do these viruses exploit the same cellular pathways to generate a productive infection? Surprisingly, of the ~1,500 transcripts affected following infection, only 32% were common to PRV and HSV-1. Key observations included changes in oxidative-stress gene transcription late in both PRV and HSV-1 infection — which could underline the important role of managing oxidation in a productive viral infection. Strikingly, heat-shock-stress genes were also affected late in infection. Perhaps the host cell sounds the alarm to galvanize the immune system into action to prevent virus spread. The P13K/Akt signalling pathways were both affected by PRV and HSV-1. These pathways encode proteins that can aid cell survival or activate apoptosis, and balancing their activity could fine-tune the outcome of virus infection. Surprisingly, while HSV-1 modulated the expression of interferon- and interleukin-regulated genes, PRV had no effect on the same pathways, which might be an important clue to help to delimit the pathogenic strategies of these viruses in rodents. One caveat is that the transcriptome only tells us about RNA levels, but both PRV and HSV-1 express proteins that regulate mRNA stability, transport and translation, which could affect protein production.

What about transcriptional responses to α-herpesvirus infection in other cell types? The rodent cell data for HSV-1 infection were compared with data gathered following infection of human cells with HSV-1 in a different study. A core set of 29 genes that were affected in both studies was defined. Of these, 12 genes were also regulated during PRV infection of rodent cells. Does this represent an α-herpesvirus host-cell signature?

Responses to viruses early in infection could prevent a productive infection from developing. But late responses might also be important. After all, the virus must spread to cause disease. This comparative study lays the foundation for an examination of α-herpesvirus-regulated genes, the differences between permissive and non-permissive hosts, and the role of late host responses in virus infection.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Ray, N. & Enquist, L. W. Transcriptional response of a common permissive cell type to infection by two diverse α-herpesviruses. *J. Virol.* **78**, 3489–3501 (2004)

WEB SITE

Lynn Enquist's laboratory:
http://www.molbio.princeton.edu/labs/enquist/