CELLULAR MICROBIOLOGY

Rafts shape *Candida*

Lipid microdomains, known as rafts, are regions of asymmetrically distributed lipids that are thought to induce cell polarity and migration in many eukaryotic cell types. Research just published in *Eukaryotic Cell* identifies a role for lipid rafts in hyphal growth of the opportunistic human pathogen *Candida albicans*.

In *Saccharomyces cerevisiae* rafts localize to the tips of pheromone-induced mating projections and might contribute to their growth, so a role for rafts in the polarized growth of other fungi seemed likely. *C. albicans* is a superb model for cell growth and morphogenesis because it has different cell morphologies — buds, pseudohyphae and hyphae — that are readily manipulated *in vitro*. Switching between buds and hyphae is also clinically relevant because hyphae can invade tissues and *C. albicans* virulence factors are hyphal-specific.

Using fluorescence microscopy and a fluorescent antibiotic (filipin) that binds to the main membrane sterol (ergosterol) Martin and Konopka observed intense staining at the tips of *C. albicans* hyphae, indicating that membrane lipids are enriched to form rafts. Tip morphology does not have a role in providing a scaffold to recruit lipids into rafts because the rafts formed even when tip shape was altered by the inhibition of hyphal growth. Germ-tube switching — the sequential formation of germ tubes that abort growth before a mature hypha is produced — can occur in *C. albicans*. Rafts were only found at the actively growing hyphal tip after germ-tube switching, so rafts only participate in active polar growth. Detergent-resistant membranes could be isolated from buds and hyphae, but raft polarization was again only detected at hyphal tips. Finally, monitoring raft location during hyphal growth from unbudded cells confirmed that rafts were only present in actively growing sites, including regions of hyphal growth initiation, the hyphal tip and at the presumptive septum just prior to septum formation.

VIRAL IMMUNE EVASION

Innate escape

The development of some viral infections in immunosuppressed individuals could be caused by selection pressure from the innate immune system driving the evolution of viral escape mutants, according to a paper in the June issue of *Immunity*.

The murine cytomegalovirus (MCMV) infection model has been an extremely useful tool to analyse the immune response to viral infection. Previous research had already demonstrated that C57BL/6 (B6) mice are resistant to MCMV infection. This resistant phenotype is linked to the *Cmv1* locus, which encodes Ly49H, an activation receptor found on the surface of natural killer (NK) cells, an important component of the innate immune response. Ly49H recognises a viral gene product, m157, which is found on the surface of MCMV-infected cells.

Analysis of the survival curves of MCMV-infected B6 severe combined immunodeficiency (SCID) mice — mice without an adaptive immune response — revealed an interesting phenomenon. In these mice, late deaths (up to 5 weeks post-infection) were observed, even at a low inoculum of MCMV; this was in contrast to the early deaths seen in NK cell-deficient susceptible mice. Additionally, analysis of the viral titre in the spleen revealed that the viral load in the SCID mice was higher 28 days after infection than it was 3 days after infection.

When plaque-purified virus isolated from the spleens of surviving B6 SCID mice (SCID-MCMV) was used to infect B6-SCID mice, high mortality was seen within 7 days. Additionally, 70–100% mortality was acutely observed when SCID-MCMV isolates were used to infect wild-type B6 mice, compared with 100% survival with wild-type MCMV infection.

These and other results suggested that the observed effect was caused by an alteration in the MCMV virus. The presence of viral escape mutants was confirmed by sequencing the viral m157 gene — mutations were identified in this gene in most of the splenic SCID-MCMV isolates analysed, whereas mutations were not found in other viral genes examined. Moreover, a deliberate mutation in m157 produced a virus with a similar phenotype.

This paper provides evidence that in the absence of an adaptive response, the selective pressure from the innate immune system can drive the evolution of escape mutants of a double-stranded DNA virus and, somewhat surprisingly, this can occur during the course of a single infection. Previously, escape mutants have mainly been found in RNA viruses, and generally as a response to adaptive immunity. These latest observations have obvious clinical implications, as they raise the possibility that the severe viral infections often seen in patients with an impaired adaptive immune response, such as in AIDS, could be caused by viruses escaping from innate immunity.

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER French, A. R. *et al.* Escape of mutant double-stranded DNA virus from innate immune control. *Immunity* **150**, 747–756 (2004)

Septins, important in sequestering mating proteins in *S. cerevisiae* mating projections, co-localized with rafts in *C. albicans*, as shown by microscopy, but were not found in detergent-resistant membranes, so a clear role for these proteins in defining raft borders cannot yet be assigned. Disruption of actin, an important cytoskeletal component known to be involved in hyphal growth, prevented raft polarization to hyphal tips.

Cell-wall-biogenesis proteins and adhesins in *C. albicans* are glycosylphosphatidylinositol (GPI)-anchored and GPI-anchored proteins are often enriched in rafts. This intriguing research therefore might indicate that raft polarization not only promotes hyphal growth but might also enrich virulence factors at invading hyphal tips during infection.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Martin, S. W. & Konopka, J. B. Lipid raft polarization contributes to hyphal growth in *Candida albicans*. *Eukaryot. Cell* **3**, 675–684 (2004)

WEB SITE

James Konopka's laboratory: http://www.uhmc.sunysb.edu/microbiology/Konopka.html

ANTI-INFECTIVES

Stuck in a tunnel

The peptide antibiotic Microcin J25 (MccJ25) binds inside a channel in the bacterial RNA polymerase (RNAP) — the secondary channel, NTP uptake channel or pore — to prevent nucleotide uptake and inhibit transcription, according to two reports just published in *Molecular Cell*.

MccJ25 is a 21-amino-acid antibiotic with an unusual lariat (knot) structure. It inhibits Gram-negative bacteria by targeting the bacterial RNAP, which is a complex of five subunits ($\alpha_2\beta\beta'\omega$). Mutations conferring resistance to MccJ25 map to the gene that encodes the β′ subunit. The use of RNAP inhibitors, including antibiotics such as rifampicin, is an elegant tool for defining mechanisms of transcription. Now, groups led by Richard Ebright and Konstantin Severinov have used complementary approaches to address how MccJ25 inhibits RNAP.

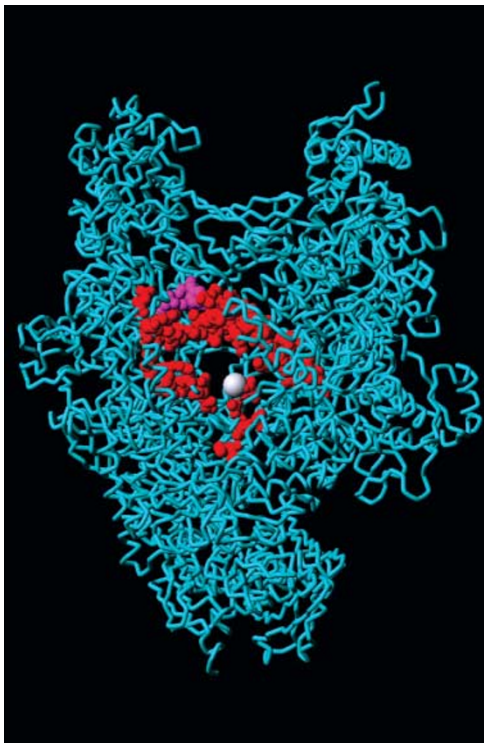
Transcription in bacteria is a multi-step process; first, RNAP binds to promoter DNA and forms a closed complex; second, the DNA is locally unwound (melted), yielding an open complex; third, transcription is initiated, producing very short RNA fragments, less than 9–11 bp (called abortive initiation); and, finally, the RNAP is released to produce a full-length transcript. By incorporating MccJ25 into assays that assemble functional RNAP–DNA complexes both groups showed that the antibiotic does not inhibit the first two steps in this process — but specifically inhibited abortive initiation and elongation, and, moreover, adding excess NTPs overcame this inhibition.

NTPs access the RNAP catalytic centre through the secondary channel, a fully enclosed tunnel that is 30 Å long and 10–15 Å wide. Genetic approaches from the Severinov group had previously pinpointed the binding site of MccJ25 to the RNAP secondary tunnel. Here, Mukhopadhyay *et al.* used saturation mutagenesis of the genes encoding the β and β′ subunits. Mutations conferring resistance to MccJ25 mapped to all the channel surfaces, but 90% of mutations were in the β′ subunit. Biochemical approaches taken by both groups revealed that NTPs and MccJ25 bind to the RNAP at the same time but that MccJ25 reduces the affinity of the RNAP–NTP interaction. Adelman *et al.* showed that MccJ25 affects RNAP-backtracking and Gre factor (transcription cleavage factors that bind in the secondary channel) cleavage, proving that it binds in the tunnel.

Biophysical techniques are precision tools for dissecting molecular processes. Mukhopadhyay *et al.* used fluorescence resonance energy transfer (FRET), together with competitive FRET (using a Gre factor and MccJ25) and systematic FRET to pinpoint the binding site of MccJ25 in the RNAP secondary channel. Adelman *et al.* adopted a sophisticated single-molecule optical-trapping technique to monitor transcription of individual RNAP molecules in real-time. When MccJ25 binds to an elongating RNAP, transcription ceases — as opposed to simply slowing down. The 'all-or-nothing' response of the RNAP to the inhibitor indicates a 1:1 RNAP:MccJ25 stoichiometry that leads to complete inhibition of RNA synthesis.

Finally, both groups docked the RNAP and MccJ25 structures — the inhibitor completely blocks the tunnel. These exciting findings could enable the rational design of new anti-infectives to combat both Gram-negative and Gram-positive bacterial pathogens.

Susan Jones



Sites of single-residue substitutions in RNAP that confer MccJ25 resistance are shown in red (β′ subunit) and pink (β subunit). The RNAP active centre Mg^{2+} is shown in white. Courtesy of Richard Ebright, Rutgers University, USA.

References and links

ORIGINAL RESEARCH PAPERS

Mukhopadhyay, J. *et al*. Antibacterial peptide Microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel. *Mol. Cell* **14**, 739–751 (2004) | Adelman, K. *et al*. Molecular mechanism of transcription inhibition by peptide antibiotic Microcin J25. *Mol. Cell* **14**, 753–762 (2004)