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.

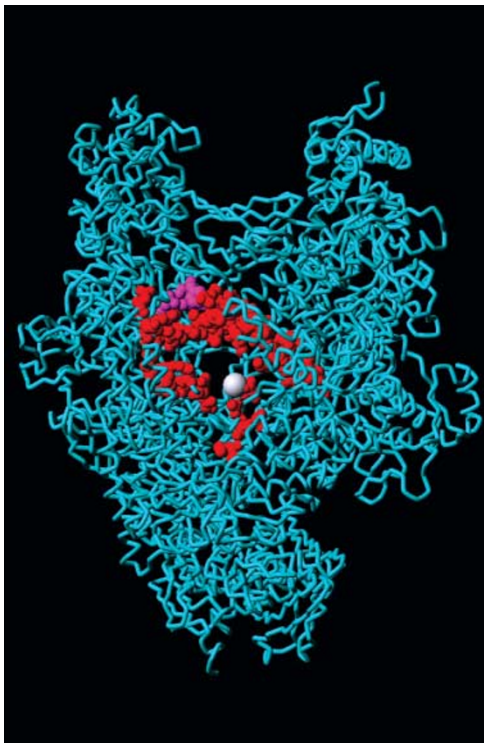
Transcription in bacteria is a multi-step process; first, RNAP binds to promoter DNA and forms a closed complex; second, the DNA is locally unwound (melted), yielding an open complex; third, transcription is initiated, producing very short RNA fragments, less than 9–11 bp (called abortive initiation); 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)