INNATE IMMUNITY

It all ends in tears

New research in *Antimicrobial Agents and Chemotherapy* presents evidence that tear lipocalin (TL) could contribute to innate immunity by scavenging siderophores and thereby inhibiting bacterial and fungal growth.

The cornea acts as a physical barrier to protect the rest of the eye. As the cornea contains no blood vessels, nutrients are provided by tear fluid, which also contains a variety of small molecules with antibacterial functions, including human tear lysozyme. TL is another component of human tear fluid, but until now its exact function has been unknown.

TL is a member of a large protein family known as the lipocalins, secreted molecules that are found in a wide variety of species and which are known to bind small hydrophobic molecules. It had previously been suggested that TL binding to these molecules could have a general protective function. In this work, Fluckinger *et al.* wanted to have a more detailed look at the function of TL.

Fluckinger *et al.* first analysed the binding of TL to different siderophores using a competitive displacement assay. There are two main groups of bacterial siderophores: the catecholates such as *Escherichia coli* enterobactin and the hydroxamates such as *Streptomyces* deferroxamine. Both these groups were analysed, along with fungal siderophores including *Aspergillus nidulans* triacetylfusarinine C. The results showed that TL bound with high affinity to all the siderophores tested with the exception of *Pseudomonas aeruginosa* pyoverdine — the authors suggest that this reflects the fact that pyoverdine has an additional chromophore residue that precludes binding in the TL active site.

Fluckinger *et al.* went on to look at the effects of TL on bacterial and fungal growth. Under non-iron-limiting conditions, the presence of TL had no effect on *E. coli* growth. However, under iron-limiting conditions, the presence of TL severely inhibited

PROTOZOAN PARASITES

Ordered domains key to *Entamoeba* virulence

A report in the latest issue of *Infection and Immunity* presents evidence that not only are lipid rafts present in the membrane of the protozoan parasite *Entamoeba histolytica* but they also contribute to the virulence of this pathogen.

The view of the plasma membrane as a homogeneous structure was dispelled long ago with the 'fluid mosaic' concept, and further modified more recently with the discovery of lipid rafts — tightly ordered, cholesterol- and glycosphingolipid-enriched microdomains, which, although the methods for their detection remain controversial, have been shown to be involved in many vital cellular functions, including signal transduction, adhesion and secretion. *E. histolytica* is the causative agent of amoebic dysentery and estimates suggest that up to 100,000 people die each year and some 50 million people are symptomatically infected, almost all in developing countries. After ingestion of cysts in contaminated food or water, *E. histolytica* excystation occurs in the small intestine, releasing trophozoites that migrate to the large intestine, adhere to the colonic mucus mainly through the interaction of a galactose/*N*-acetyl galactosamine (Gal/GalNAc)-specific lectin with host glycoconjugates and then obtain nutrients via the endocytic pathway.

To investigate whether the *E. histolytica* membrane contained raft-like domains, Laughlin *et al.* stained trophozoites with two lipid analogues — one ($DiIC_{16}$) that preferentially partitions into ordered domains and another (FAST-DiI) that partitions into more fluid domains. The staining pattern indicated that tightly ordered domains were indeed present. To aid further interpretation of the staining pattern, the experiment was repeated but the trophozoites were first treated with raft-disrupting agents that remove (MBCD) or sequester (Filipin) cholesterol from the membrane. This treatment affected $DiIC_{16}$ but not FAST-DiI binding, suggesting that $DiIC_{16}$ co-localized with cholesterol-rich, raft-like domains.

To investigate the physiological function of the rafts, Laughlin *et al.* went on to look at the effects of raft-disrupting agents on pinocytosis (the uptake of fluids or solutes), secretion and adhesion to host cells. MBCD treatment significantly inhibited fluid-phase pinocytosis but had no effect on the secretion of cysteine proteases, suggesting that raft-like domains are involved in pinocytosis but that the secretion of cysteine proteases is raft independent. MBCD treatment also inhibited the adhesion of trophozoites to a mammalian cell monolayer and further work revealed that the Gal/GalNAc-specific lectin that has an important role in *E. histolytica* adhesion is enriched in cholesterol-containing membrane fractions; these results suggest that the adhesion of *E. histolytica* to host cells is also raft dependent.

This work is the first report of the presence of lipid rafts in the *E. histolytica* membrane. The authors hope that future analysis of these regions will provide a further insight into *E. histolytica* pathogenesis.

Sheilagh Clarkson

E. coli growth and this inhibition was reversed by the addition of free iron. In agreement with previous results, the presence of TL had no effect on *P. aeruginosa* growth. For fungi, the authors examined the effect of TL on the growth of a mutant strain of *A. nidulans* that is deficient in siderophore biosynthesis and showed that the presence of TL significantly inhibited growth.

One member of the lipocalin family, neutrophil gelatinase-associated lipocalin (NGAL), was already known to scavenge siderophores. This paper shows that TL also mops up siderophores, thereby inhibiting bacterial growth; unlike NGAL, however, which only binds to bacterial catecholate siderophores, TL binds to both catecholate and hydroxamate siderophores, and can inhibit both bacterial and fungal growth.

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER Fluckinger, M. *et al*. Human tear lipocalin exhibits antimicrobial activity by scavenging microbial siderophores. *Antimicrob. Agents Chemother*. **48**, 3367–3372 (2004)

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ORIGINAL RESEARCH PAPER Laughlin, R. C. *et al*. Involvement of raft-like plasma membrane domains of *Entamoeba histolytica* in pinocytosis and adhesion. *Infect. Immun*. **72**, 5349–5357 (2004)

FURTHER READING Huston, C. D. Parasite and host contributions to the pathogenesis of amebic colitis. *Trends Parasitol*. **20**, 23–26 (2004)

CELLULAR MICROBIOLOGY

Ping-pong cell-fate control

"Dr Jacobs-Wagner has observed a critical step in how cells control their development. Although her discovery was made in a…bacterium, it's likely that similar processes also control development in higher organisms, including humans. These findings will likely lead to a better understanding of both normal and abnormal human development" said Marion Zatz, Ph.D., chief of the developmental and cellular processes branch at the National Institute of General Medical Sciences, which partially funded the work.

New research published in *Cell* has linked initiation of a developmental programme in the model bacterium *Caulobacter crescentus* to the completion of cytokinesis.

Generating different cell types isn't just important for some bacteria — in development, eukaryotes use asymmetric cell division to generate a diverse array of cell types. *Caulobacter* divides asymmetrically at each cell division to produce a sessile stalked cell and a smaller motile swamer cell. The swarmer cell undergoes a swarmer-progeny-specific (SwaPS) developmental programme to differentiate into a stalked cell. Inhibition of cytokinesis blocks the initiation of SwaPS development.

Two histidine kinases, DivJ and PleC, which function through the shared response regulator DivK, had already been implicated in coupling SwaPS development with cell division. DivJ phosphorylates DivK, while PleC promotes DivK dephosphorylation, either directly or indirectly. All three regulators are localized in the *Caulobacter* cell — PleC at the flagellar pole, DivJ at the stalked pole and DivK at both poles, with the localization of DivK modulated by DivJ and PleC. After cytokinesis, DivK is no longer present at the flagellar pole. The latest report from the Jacobs-Wagner laboratory examines how these proteins are localized to specific locations and whether localization affects SwaPS development.

Mutating PleC disrupts SwaPS development, possibly by preventing DivK release from the flagellar cell pole. A mutant form of DivK ($DivK_{D90G}$) that suppressed a *pleC* knockout mutant was not localized at the flagellar pole of the nascent swarmer cell. By depleting FtsZ, and therefore disrupting cell division, Matroule *et al*. showed that DivK–GFP was only released from the flagellar pole when cell division proceeded. If DivK is an intrinsic part of the control mechanism it should be possible to undergo SwaPS without cytokinesis if DivK is not present at the flagellar pole — this was the case for the $DivK_{D90G}$ mutant.

If DivK release is the switch, how does the cell sense that cytokinesis is complete and release DivK from the flagellar pole? Using a mutant form of PleC, which had phosphatase activity but lacked kinase activity, Matroule *et al*. showed that PleC is primarily a DivK~P phosphatase *in vivo*. Fluorescence resonance energy transfer (FRET) analysis showed that DivJ and PleC physically interact with DivK at the poles — DivJ/DivK interact at the nascent stalked cell pole, and PleC/DivK interact at the nascent flagellar pole. Finally, fluorescence photobleaching revealed that DivK shuttles from one pole to the other by diffusion. *Caulobacter* monitors communication between the cell poles through 'ping-pong' shuttling of DivK and DivK~P.

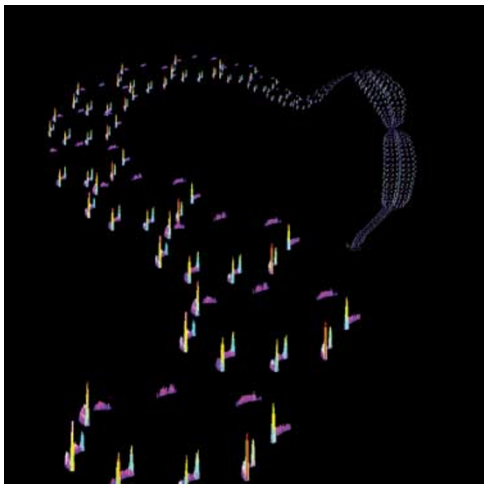

Image courtesy of Christine Jacobs-Wagner.

Keeping DivK~P at the flagellar pole inhibits SwaPS development. Cytokinesis separates the daughter cells, preventing DivK~P (produced by DivJ kinase activity at the stalked pole) from shuttling to the flagellar pole. DivK~P at the flagellar pole is completely dephosphorylated by PleC and released into the cytoplasm, and SwaPS development proceeds, but how DivK itself controls cell fate still needs to be resolved.

In *Caulobacter*, cytokinesis triggers cell development — this might be the first true bacterial checkpoint to be identified. It might also be a paradigm for developmental controls that function at cell poles from bacteria to man.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Matroule, J.-Y. *et al*. Cytokinesis monitoring during development: rapid pole-to-pole shuttling of a signaling protein by localized kinase and phosphatase in *Caulobacter*. *Cell* **118**, 579–590 (2004)

WEB SITE

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