BACTERIAL PHYSIOLOGY

Pinpointing the pathway

Researchers have identified a missing metabolic pathway that has turned out to be the most widely distributed acyltransferase system in bacteria, according to a recent report in *Molecular Cell*.

Phosphatidic acid is a key intermediate in the synthesis of membrane phospholipids. In the model Gram-negative organism *Escherichia coli*, the first step in the formation of phosphatidic acid is the acylation of glycerol-3-phosphate (G3P). This step is carried out by PlsB, a G3P acyltransferase that uses either acyl-ACP (ACP is an acyl-acyl carrier protein) or acyl-coenzyme A to add an acyl group to G3P. However, Gram-positive bacteria do not produce PlsB and in some Gram-negative organisms, *plsB* is non-essential, indicating that there must be another pathway capable of carrying out this key reaction.

Ying-Jie Lu and colleagues began investigating phosphatidic acid synthesis in the Gram-positive organism *Streptococcus pneumoniae*. Previous work in *E. coli* had identified another protein, PlsX, which it was thought might be involved in G3P metabolism. Biochemical analysis of the activity of *S. pneumoniae* PlsX showed that it had no intrinsic G3P-acyltransferase or fatty-acid kinase activity. Rather, it was found that *S. pneumoniae* PlsX is a soluble phosphotransacylase that catalyses the reversible conversion of the end product of type II fatty-acid biosynthesis, acyl-ACP, to a fatty-acyl phosphate, a new metabolic intermediate.

The authors then wondered whether there could be a second protein that used this activated fatty-acid intermediate to generate acylated G3P. They analysed the acyl-phosphate-dependent G3P-acylation activity in *S. pneumoniae* and found that, in contrast to *E. coli*, the main *S. pneumoniae* G3P acyltransferase activity requires an acyl-phosphate as the acyl donor. The protein responsible for this activity in *S. pneumoniae* was identified as the membrane-associated protein PlsY.

Analysis of the distribution of *plsX* and *plsY* revealed that the PlsX/Y pathway is widespread, with *plsX* and *plsY* present in 314 of the 395 complete and draft sequences examined. By contrast, *plsB* was only found in 102 of the 395 genomes examined, and was mainly confined to the γ-proteobacteria. So, it seems that Lu *et al.* have identified the main pathway responsible for the first step of phosphatidic acid biosynthesis in bacteria.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Lu, Y-J. *et al.* Acyl-phosphates initiate membrane phospholipid synthesis in Gram-positive pathogens. *Mol. Cell* **23**, 765–772 (2006)

VIRUSES AND CANCER

Viral hijacking

Despite its association with tumours and unlike other DNA viruses, human cytomegalovirus (HCMV) does not transform cells, so how it exerts its oncogenic potential has been unclear. Martine Smit and colleagues now show that *US28*, an HCMV gene that encodes a G-protein-coupled receptor, is important for initiating angiogenesis in transformed cells.

The US28 receptor is a homologue of the human chemokine receptor CCR1 but, unlike its cellular counterpart, it signals in a constitutively active manner and has previously been shown to activate pathways that lead to cell proliferation and migration.

HCMV might use US28 to hijack several signalling networks within infected cells. In fact, this viral receptor displays promiscuous G-protein coupling that causes the constitutive activation of different G proteins and potentiates the signalling of other cellular chemokine receptors.

Smit and colleagues found that when US28 is stably expressed in NIH-3T3 cells, the cells have an increased growth rate that results, at least in part, from the increased expression of cyclin D1. The US28-expressing cells also form foci *in vitro*, and after 5 days the cells express high levels of vascular endothelial growth factor (VEGF) compared with cells that express a mutant US28 that is unable to bind G proteins.

To further dissect the pathway activated by US28, the authors investigated the downstream signalling pathways and found that US28

PLANT DISEASE RESISTANCE

An open and shut case

A recent report in *Cell* has revealed that rather than being passive ports of entry, stomata have an active role in the innate immune response of plants to bacterial challenge, and that bacteria have evolved specific virulence factors to counteract this defence reaction.

Unlike fungal pathogens, bacterial pathogens cannot penetrate plant tissues directly. Instead, they rely on entry though wounds or natural openings in the plant surface. Stomata are microscopic pores in the plant epidermis that allow gaseous exchange and transpiration. Each stomatal pore is surrounded by two guard cells that control stomatal opening and closing in response to changing environmental conditions, such as light and humidity.

In this work, Maeli Melotto and colleagues studied the response of *Arabidopsis thaliana* stomata to challenge with the plant pathogen *Pseudomonas syringae* pv. tomato DC300 (*Pst* DC300). After 1 hour of incubation with *Pst* DC300, the average width of the stomatal aperture decreased dramatically, and after 2 hours the number of open stomata was reduced by ~70%.

Is stomatal closure stimulated by a specific bacterial component, such as a PAMP (pathogen-associated molecular pattern)? Melotto *et al.* found that two well-known PAMPs — a flagellin-derived peptide and lipopolysaccharide — stimulated stomatal closure. In *A. thaliana*, the innate immune response can be either salicylic acid (SA)-dependent or SA–independent and the PAMP response was found to be part of the SA-dependent pathway. Stomatal closure is regulated by an abscisic acid (ABA) signalling pathway in guard cells, and Melotto *et al.* found that the PAMP-induced closure also involved ABA signalling.

The induction of stomatal closure was not restricted to *Pst* DC300 and was also observed with the human pathogen *Escherichia coli*

signals through both Gαq and Gβγ protein subunits and two different MAPKs (mitogen activated protein kinases) to stimulate downstream transcription factors, which ultimately lead to *VEGF* promoter activation. Consistent with this, NIH-3T3 cells that expressed US28 formed highly vascularized, VEGF-expressing tumours 2 weeks after inoculation into nude mice. This indicates that VEGF-mediated angiogenesis is responsible for at least some of the oncogenic properties of US28.

To verify these findings the authors used an HCMV strain that does not express US28 to infect a glioblastoma cell line. This strain failed to induce *VEGF* promoter activation, unlike the wild-type virus.

Interestingly, the expression of US28 in non-tumorigenic cells can also induce apoptosis. So, it seems that the cellular context determines whether *US28* functions as an oncogene and a pro-angiogenic factor. The authors conclude that US28 might be a potential target for the treatment of early-stage HCMV-related proliferative diseases.

Francesca Pentimalli
Nature Reviews Assistant Editor

ORIGINAL RESEARCH PAPER Maussang, D. *et al.* Human cytomegalovirus-encoded chemokine receptor US28 promotes tumorigenesis. *Proc. Natl Acad. Sci. USA* **103**, 13068–13073 (2006)
FURTHER READING Damania, B. Oncogenic γ-herpesviruses: comparison of viral proteins involved in tumorigenesis. *Nature Rev. Microbiol.* **2**, 656–668 (2004).

O157:H7. Interestingly, the length of the induced closure differed between these two pathogens — with *E. coli* O157:H7, closure persisted for the 8-hour duration of the experiment, whereas *Pst* DC300-induced closure was reversed after ~3 hours. This suggested that *Pst* DC300 might have evolved a mechanism to reopen the stomata. *Pst* DC300 has two main virulence factors, a type III secretion system (T3SS) and the phytotoxin coronatine. Analysis of the response to *Pst* DC300 mutants deficient in coronatine or with a defective T3SS demonstrated that coronatine is the virulence factor involved in suppressing stomatal closure, and it was shown to function downstream of ABA.

So, in addition to their key role in gaseous exchange and transpiration, plant stomata also function as 'innate immune gates'. Rather than being able to freely enter plant tissues through the stomata, *Pst* DC300 triggers initial stomatal closure through the detection of PAMPs and the ABA signalling pathway. The bacteria then counteract this defence response by secreting coronatine, which causes the stomata to reopen. Given that stomata are present in all vascular plants, the authors speculate that PAMP-induced stomatal closure could be a widespread phenomenon and that the inhibition of this defence response might have been a key adaptation in the evolution of plant pathogens.

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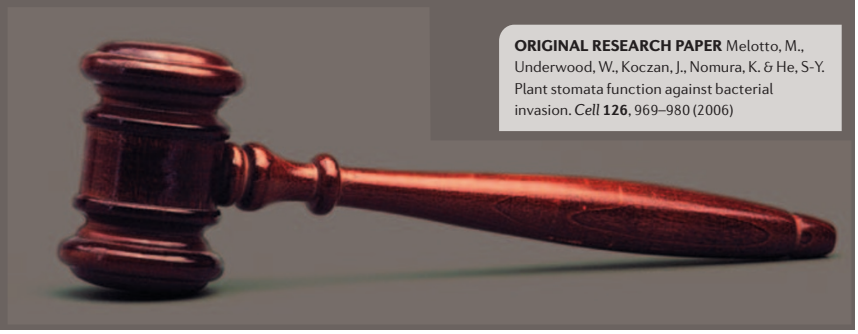

ORIGINAL RESEARCH PAPER Melotto, M., Underwood, W., Koczan, J., Nomura, K. & He, S-Y. Plant stomata function against bacterial invasion. *Cell* **126**, 969–980 (2006)

IN BRIEF

SYMBIOSIS

Symbiosis insights through metagenomic analysis of a microbial consortium

Woyke, T. *et al. Nature* 17 September 2006 (doi:10.1038/nature05192)

Nicole Dubilier, Edward Rubin and colleagues report in a recent issue of *Nature* on their metagenomic analysis of the bacterial endosymbionts present under the cuticle of the marine oligochaete worm *Olavius algarvensis*. Using shotgun sequencing and metabolic pathway reconstruction, Woyke *et al.* were able to characterize four bacterial cosymbionts. The bacteria are either γ- or δ-proteobacteria, and can generate energy by carbon fixation and either oxidization of sulphides or reduction of sulphates. Other detailed metabolic information obtained allowed the authors to reconstruct the physiology of two of the symbionts and their interactions with the worm.

BACTERIAL PATHOGENICITY

Virulence factors of *Yersinia pestis* are overcome by a strong lipopolysaccharide response

Montminy, S. W. *et al. Nature Immunol.* **7**, 1066–1073 (2006)

Yersinia pestis can undergo temperature-dependent alterations in its lipopolysaccharide (LPS). At 21–27°C, the average temperature of the flea vector, the lipid A component of LPS is hexa-acylated whereas at 37°C, mammalian body temperature, it is tetra-acylated. It had previously been suggested that this temperature-dependent switch could be involved in immune evasion, as tetra-acylated LPS is a poor stimulator of Toll-like receptor 4 (TLR4). Montminy *et al.* expressed the gene encoding the *Escherichia coli* LpxL acyltransferase in *Y. pestis*. The presence of this gene caused *Y. pestis* to synthesize hexa-acylated LPS, which was a potent TLR4 stimulator, but this strain of *Y. pestis* (KIM1001-pLpxL) was avirulent in mice. Resistance to KIM1001-pLpxL was found to require the presence of TLR4 and the TLR4 adaptor MyD88 and co-receptor MD-2. These results indicate that the virulence of *Y. pestis* is strongly dependent on the evasion of the LPS–TLR4 signalling pathway.

QUORUM SENSING

Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing

Neiditch, M. B. *et al. Cell* **126**, 1095–1108 (2006)

The quorum-sensing signal autoinducer 2 (AI-2) is produced and detected by both Gram-negative and Gram-positive species. In *Vibrio harveyi*, the AI-2 receptor comprises LuxP and LuxQ, a periplasmic protein and membrane sensor histidine kinase, respectively. Neiditch and colleagues present the crystal structures of the periplasmic domain of LuxQ ($LuxQ_p$) and of $LuxPQ_p$ bound to AI-2. The most notable ligand-induced structural change is a major conformation change in LuxP — AI-2 binding promotes the dimerization of the LuxP periplasmic regions, generating asymmetric $LuxPQ_p$ dimers. The authors propose a model in which, in the absence of AI-2, the interaction between LuxP and LuxQ forms a 'clasp' between the proteins, and in the presence of AI-2, this clasp is released.