BACTERIAL GENETICS

MutS2: key to diversity?

Studies in the gastric pathogen *Helicobacter pylori* have at last identified the function of the bacterial MutS2 proteins, according to results reported in a recent *Molecular Cell* paper.

H. pylori is one of the most genetically diverse bacterial species examined to date, and the plasticity of the *H. pylori* genome has been the topic of much research over the past 5–6 years. In the gastric mucosa, genetic material is frequently exchanged between bacteria, and recombination is believed to be a key mechanism of generating genetic diversity in *H. pylori*.

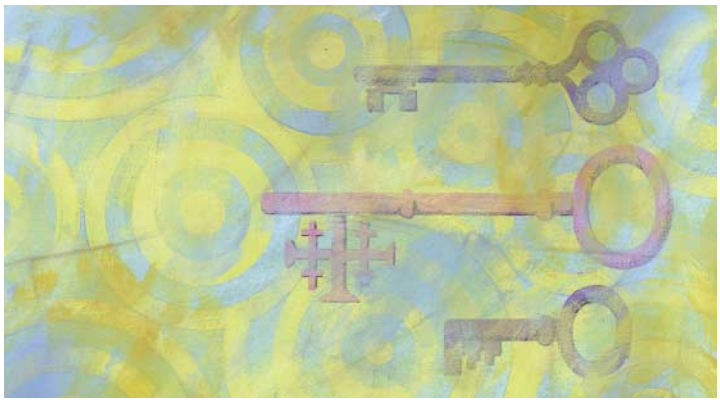
In *Escherichia coli*, the MutS protein specifically recognizes mismatched nucleotides and therefore has a key role in post-replication mismatch repair and the removal of mismatches generated during recombination. Two families of MutS homologues are known, the MutS-I family, which includes *E. coli* MutS, and the MutS-II family. The only evidence for a putative mismatch repair system in *H. pylori* is the identification of a single open reading frame, HP0621, which encodes MutS2, a MutS homologue that belongs to the MutS-II family.

Until now, the function of the bacterial MutS2 proteins has been unknown. Pinto *et al.* first investigated whether *H. pylori* MutS2 has a role in mismatch repair by disrupting the *mutS* gene in *H. pylori* with a variety of genetic backgrounds and comparing the rates of spontaneous mutation in an essential locus (*rpoB*, conferring rifampicin resistance) and a non-essential locus (*rdxA*, conferring metronidazole resistance) with the rates observed in wild-type bacteria. Disruption of *mutS* had little or no effect on the mutation rates and the authors therefore concluded that the *H. pylori* MutS2 protein is not involved in mismatch repair.

The MutS-II family also includes the eukaryotic MSH4 and MSH5 proteins, which are involved in meiotic recombination. If MutS2 is not involved in mismatch repair, does it have a role in homologous recombination? Pinto *et al.* monitored the incorporation of a selectable marker into a non-essential locus and showed that recombination was fivefold higher in *H. pylori* with a disrupted *mutS2* gene than in wild type. Any effects on restriction systems were ruled out, and the results were therefore consistent with a role for MutS2 in inhibiting recombination. The fact that an increased frequency of recombination was observed not only between molecules with identical sequences but also between molecules with divergent sequences indicates that MutS2 inhibits both homologous and homeologous recombination. Finally, a detailed examination of the biochemical properties of MutS2 showed that it has ATPase activity, has an affinity for structures that resemble recombination intermediates and also blocks DNA strand transfer *in vitro*.

The bacterial MutS2 proteins may therefore have a key role in the control of bacterial genetic diversity by virtue of their ability to suppress recombination. The authors conclude by highlighting the fact that these proteins "could have important consequences in the appearance of phenotypic variants and new antibiotic resistances".

Sheilagh Molloy



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PLANT PATHOGENS

Signalling complexities for *Pseudomonas*

A new paper in *Proceedings of the National Academy of Sciences USA* has uncovered a previously unknown activity of the phytopathogen *Pseudomonas syringae*: the ability to induce systemic-induced susceptibility (SIS) to subsequent infection, and has also identified the small molecule required to do so.

Generally, plants respond to an attack by microbial pathogens by inducing salicylic acid (SA)-dependent systemic resistance pathways — systemic acquired resistance (SAR) — whereas the resistance pathways induced in response to an attack by insect herbivores are jasmonic acid (JA)-dependent. It is known that the crosstalk between the two pathways, which can be additive or antagonistic, is complex and varies depending on the pathogens and plant hosts involved.

In previous work, a model system involving *Arabidopsis*, *P. syringae* and an insect herbivore, the cabbage looper (*Trichoplusia ni*), was established to investigate how infection by a bacterial pathogen affects the response of the plant to subsequent attack by other bacterial pathogens and insect herbivores. The results obtained suggested the presence of two signalling pathways, one of which was correlated with SAR and enhanced resistance to *T. ni* feeding and a second that enhanced susceptibility to *T. ni* feeding via an unknown signal.

In this latest work, infection of *Arabidopsis* lower leaves with virulent strains of *P. syringae* showed a small but reproducible SIS effect, with enhanced secondary growth of *P. syringae* in uninfected upper leaves. Further analysis pointed to the phytotoxin coronatine (COR), an important *P. syringae* virulence factor and a mimic of JA, as the molecule responsible for this effect. The authors hypothesized that the effects of COR could be mediated by antagonism of the SA-dependent SAR response. Experimental work showed that avirulent non-COR-producing strains elicited a stronger SAR response than did avirulent COR-producing strains, suggesting that COR could function by interfering with SA-dependent signalling.

The authors also analysed the effects of COR on insect herbivory, and interpret their observations as

BACTERIAL PHYSIOLOGY

Competitive signalling

N-acylhomoserine lactones, better known as AHLs, are the quorum-sensing molecules that Gram-negative bacteria use to coordinate cell-density-dependent processes, which include virulence, biofilm formation and antibiotic synthesis. A new study published in *Proceedings of the National Academy of Sciences USA* shows that these versatile molecules can also function as antimicrobials.

Studying the coordinated light production of the glowing bacterial symbionts that populate the light organ of the squid led to a realization that Gram-negative bacteria use diffusible signalling molecules to coordinate population behaviour and behave as multicellular groups. These signalling molecules, named quorum-sensing hormones or quormones, are produced by many Gram-negative bacteria. Intense research in the past 10 years has led to the elucidation of quormone biosynthetic pathways and an understanding of the regulatory functions of quormones.

AHLs, one class of quormone, are produced by several important Gram-negative animal and plant pathogens, including *Pseudomonas aeruginosa*, which can infect cystic fibrosis sufferers to cause debilitating opportunistic infections. While investigating how long AHLs can persist in an aqueous environment, Kaufmann *et al.* discovered that one of the AHLs produced by *P. aeruginosa*, *N*-(3-oxododecanoyl) homoserine lactone, was non-enzymatically and spontaneously converted into a tetramic acid. Importantly, this wasn't a peculiarity of *N*-(3-oxododecanoyl) homoserine lactone, and tetramic acids were produced by a selection of variant chain-length AHLs that Kaufmann *et al.* tested.

Using bioassays, the tetramic acid produced was shown to be cytotoxic — at concentrations that would be present in biofilms formed by *P. aeruginosa* — but only towards Gram-positive bacteria, including *Bacillus*, *Streptococcus* and *Listeria* species. Surprisingly, *N*-(3-oxododecanoyl) homoserine lactone was also mildly cytotoxic towards some of the Gram-positive strains tested. Both the AHL and the tetramic acid are much less potent than other antimicrobials in clinical use, but, nonetheless, their activity might give the producer organism a competitive advantage in a mixed community.

Tetramic acids also bind metals, although the effects of metal chelation on their cell-killing functions are variable. The AHL-derived tetramic acid identified in this study chelated Fe^{3+}, and although the tetramic acid didn't bind the metal as tightly as pyoverdin, the main siderophore of *P. aeruginosa*, it had higher affinity for iron than pyochelin, a second siderophore found in this species, so tetramic acid might function both as an iron scavenger and as an antimicrobial.

These interesting observations need to be related to the behaviour of AHL-producing bacteria in mixed communities, but it seems that when a quorum is reached, the signals that are produced might regulate multiple phenotypes and allow Gram-negative producer species to gain a competitive advantage in communities.

Susan Jones

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WEB SITE

Kim Janda's laboratory:
http://www.scripps.edu/chem/janda/

indicating that virulent *P. syringae* also induces systemic susceptibility to *T. ni* herbivory but not via COR. In fact, it was found that COR induces systemic resistance to *T. ni*, consistent with its role as a JA mimic.

So, this work has revealed a role for COR in *P. syringae*-mediated manipulation of plant systemic defences and has also confirmed that the interactions involved in these defences are extremely complex. Future work will provide further details on the role of COR and its interactions with the SA- and JA-mediated pathways, as well as continuing to analyse the molecular mechanisms responsible for *P. syringae*-mediated susceptibility to insect herbivores.

Sheilagh Molloy

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BACTERIAL PHYSIOLOGY

A function for redundancy

The availability of genome sequences promises to enable researchers to understand the physiology of individual cells, but when there are two or more pathways for metabolism of a substrate — known as metabolic modules — it takes more than 'omics to probe which pathway is used and when. Marx *et al.* used flux analysis to show that methylobacteria metabolize formaldehyde by routing it through two different pathways, and report their findings in the latest issue of *PLoS Biology*.

Methylobacteria are facultative methylotrophs, meaning that these bacteria can grow on carbon compounds that have one or more carbon atoms. Methylotrophs can grow on methanol, which is important because plants produce methanol, and plant–bacterial associations might affect seed germination and plant development.

Growing on methanol presents methylotrophs with a problem — the central intermediate in methanol catabolism is formaldehyde, which is toxic. In methylotrophs, formaldehyde can be converted into serine, and shuttled into central metabolism by one of two routes that are found only in these bacteria — a direct route (green) or a long route (blue) (see figure). The direct route is a non-enzymatic reaction that combines formaldehyde with tetrahydrofolate to generate methylene-H_4F, whereas the long route consumes one molecule of ATP and involves several enzyme-catalysed steps. A third module found in many bacteria oxidizes formaldehyde to CO_2. Genetics indicate that both the direct and the long routes are required for growth on formaldehyde, but why does *Methylobacterium extorquens* have redundant metabolic modules? Marx *et al.* have tested this directly by monitoring the metabolism of methanol using stable-isotope and radioisotope-labelling approaches.

By growing *M. extorquens* on methanol labelled with deuterium (CD_3OD) they were able to unravel which module was used in different growth conditions, since the direct route incorporates 1 deuterium atom in each serine molecule, while the long route incorporates 2 deuterium atoms in each serine. By measuring the ratios of labelled serine produced they found that metabolism of formaldehyde by the long route dominated when succinate-grown cells were first fed methanol, but that after an acclimation period the direct route was favoured. Although both routes operate, they are used differentially dependent on the growth conditions.

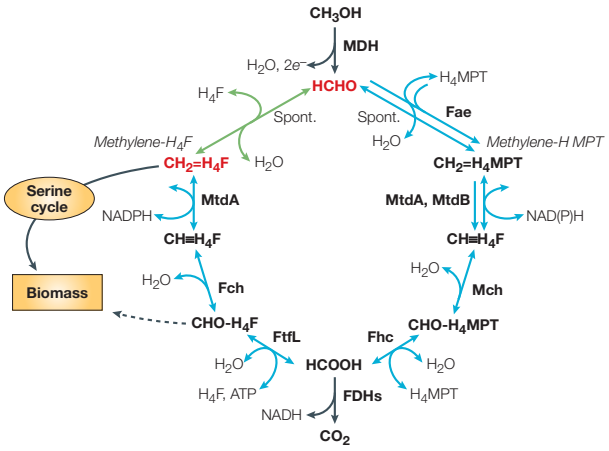

Image modified from Marx *et al. PLoS Biol.* (2005)

Flux of carbon through metabolism was also monitored using ^{14}C-labelled CH_3OH and by analysing the rates of methanol oxidation, assimilation of C1 units and CO_2 production. This analysis confirmed that the long route is initially used when cells are transferred to methanol, and that although the contribution of this route quickly declines, it is still considerable. A mathematical model produced using known kinetic parameters for the enzymes in formaldehyde metabolism was also devised. By simulating a switch from growth on succinate to methanol, the model predicted the same switch from the long to the direct route that was found using flux analysis. Marx *et al.* endeavoured to delete the *ftfL* gene, an intrinsic part of the long route, reasoning that if the long route is only necessary for acclimation to methanol growth, it might be possible to find mutants if the bacteria were already growing on methanol. The failure of this strategy indicates that both routes are needed, even when cells are continuously grown on methanol.

This research shows that biochemistry must go hand-in-hand with genomics to create useful models to understand cell physiology. Switching between different metabolic modes and metabolizing toxic intermediates isn't restricted to bacteria, so this research could represent a new paradigm for growth in toxic environments.

Susan Jones

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WEB SITE

Mary Lidstrom's laboratory: http://www.hhmi.org/research/professors/lidstrom_bio.html

BACTERIAL PATHOGENESIS

Phages and the timing of cholera

It has been 150 years since the epidemiologist John Snow traced the source of an epidemic of cholera to the Broad Street pump in London. Yet, despite Snow's acuity, certain parts of the world are still blighted by cholera epidemics that occur with an as-yet-unexplained seasonal regularity.

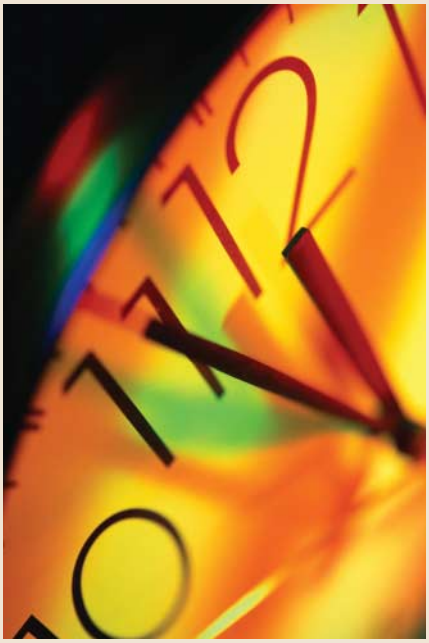
Now, the combined efforts of scientists from Bangladesh, India and the United States have revealed that epidemics of cholera are inversely correlated with the prevalence of cholera phages in contaminated water, and these findings are reported in *Proceedings of the National Academy of Sciences USA*.

John Mekalanos and co-workers analysed samples from water sources in Dhaka, Bangladesh — a city where cholera epidemics occur during certain months every year. The researchers found a statistically significant inverse correlation between the level of virulent cholera phages and that of phage-susceptible epidemic *Vibrio cholerae* strains O1 and O139 in sampled water. Importantly, when the time of onset of cholera epidemics was compared with the concentration of cholera phages in these samples, a striking pattern was observed — cholera epidemics that were caused by either the O1 or O139 serogroup strains usually commenced when low levels of O1 or O139-specific cholera phages were recovered. By contrast, a decline in the number of clinical cholera cases was associated with a marked increase in the prevalence of phages that were specific for the epidemic cholera strain.

Interestingly, during the study period, several environmental (non-pathogenic) *V. cholerae* strains were found to carry serotype-specific temperate phages, and the authors propose that the release of these phages by environmental strains might control cholera during the period between epidemics.

Finally, Mekalanos and his team propose a model in which cycles of phage amplification and predation might explain the seasonality of this disease.

Shannon Amoils



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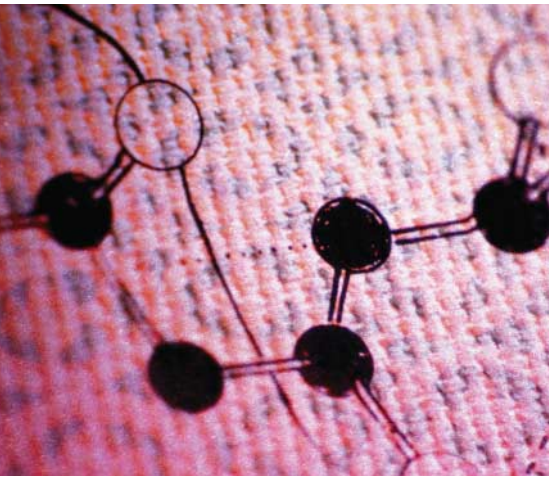
WEB SITE

International Centre for Diarrhoeal Disease Research, Bangladesh: http://www.icddrb.org/

VIROLOGY

The missing link…

A long-standing dilemma that has puzzled researchers of varicella zoster virus (VZV), the causative agent of chickenpox (varicella) and shingles (zoster), is how airborne virions that emerge from skin lesions are able to readily transmit to new hosts, yet when grown *in vitro*, the virus is highly cell-associated and very few infectious virions are released. A new study published in *Cell* resolves this issue and implicates a host protein — the mannose 6-phosphate receptor (MPR) — as being a key molecule in both processes.



Cell association of VZV has been attributed to the diversion of newly assembled virions to late endosomes where they are degraded prior to exocytosis. As previous work has shown that VZV is able to interact with cation-independent MPRs via its envelope glycoproteins, Michael Gershon and colleagues speculated that the presence of MPRs in the membrane of vesicles used to transport the newly enveloped virions could be responsible for their re-routing to late endosomes. Furthermore, previous research also demonstrated that mannose 6-phosphate, the ligand of MPRs, inhibits the infection of host cells by free VZV particles, indicating that MPRs have a role in the infection of new cells. The goal of this study was to test the hypothesis that the intracellular trafficking of newly assembled VZV and the infection of target cells by free virions are MPR-dependent.

To this end, the authors generated five human cell lines deficient in MPRs using antisense cDNA technology. Analysis of these cell lines revealed that all were resistant to infection by cell-free VZV, although the cells could be infected by cell-associated VZV. Once infected, the authors were able to demonstrate that the mutant cell lines could secrete infectious virions, thus supporting the hypothesis that both infection of naive cells by the free virus and diversion of newly assembled VZV to late endosomes require the participation of MPRs.

Further investigation of VZV infection in human epidermis revealed that the intracellular pathway of virus in superficial epidermal cells resembled that observed with the MPR-deficient cell lines. These results support the contention that, as MPR expression is lost in maturing superficial epidermal cells of the skin and VZV is not diverted to late endosomes, the virus can be secreted in a form able to propagate infection to new hosts in a controlled manner.

David O'Connell

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BACTERIAL PATHOGENESIS

Coiled coils in type III secretion

Type III secretion systems (TTSSs) are essential for the virulence of many Gram-negative pathogens; however, a detailed understanding of filament formation and type III protein translocation has remained elusive. Now, a report in *Nature Structural and Molecular Biology* describes the high-resolution structure of the TTSS filament protein of enteropathogenic *Escherichia coli* (EPEC), EspA, in complex with its secretion chaperone, CesA, providing new insights to these fundamental pathogenic processes.

One of the best-characterized TTSSs is that of EPEC, in which the EspA protein polymerizes to form the needle-like extracellular filament through which the bacteria can inject virulence proteins directly into the eukaryotic cell cytoplasm. However, structural analysis of EspA has been difficult due to the propensity of EspA to polymerize and form filamentous multimers. In addition, expression of EspA is controlled post-transcriptionally, which highlights the need to prevent premature polymerization upon synthesis and poses questions regarding the mechanisms of filament formation.

Following a detailed biochemical analysis of CesA, Calvin Yip, a Ph.D. student in the laboratory of Natalie Strynadka, discovered that this secretion chaperone protein inhibits polymerization of EspA, and used this information to crystallize a heterodimeric CesA–EspA complex, which diffracted to high resolution. CesA was found to have an 'all-helical' topology — a long N-terminal α-helix (α1) and two shorter C-terminal α-helices (α2 and α3). EspA was also largely helical, consisting of a long N-terminal α-helix (αI) and a C-terminal α-helix (αII). The central region of EspA is likely to be flexible as no clear electron density could be observed. The two proteins interact through a long coiled-coil between helix α1 of CesA and helix αII of EspA. This coiled-coil interaction is believed to also be important for EspA–EspA interactions, as deletion mutagenesis has shown the EspA αII helix to be essential for both CesA–EspA and EspA–EspA interactions.

The authors found that CesA binds to monomeric but not polymerized EspA, and thermal denaturation experiments indicate that the heterodimeric CesA–EspA complex is thermodynamically more stable than the CesA dimer. This led them to propose a mechanism whereby newly expressed EspA monomers displace CesA from a CesA homodimer, so that monomeric EspA but not oligomeric forms are transported to the bacterial surface, and EspA monomers are secreted with the α-helices necessary for filament formation intact, ensuring efficient filament formation.

Jane Saunders

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WEB SITE

Natalie Strynadka's laboratory: http://byron.biochem.ubc.ca/

IN BRIEF

PRIONS

Neuroinvasion by scrapie following inoculation via the skin is independent of migratory Langerhans cells

Mohan, J. *et al. J. Virol.* **79**, 1888–1897 (2005)

In scrapie, the abnormal prion protein (PrP^{Sc}) accumulates in follicular dendritic cells in lymphoid tissue germinal centres, before translocating in the peripheral nerves from the lymphoid tissues to the central nervous system. Scrapie has been shown to be transmitted through the skin but the mechanism of transport of PrP^{Sc} from the skin to the lymphoid tissues is unknown. Here, Mohan and colleagues, using a murine model of scrapie, studied the role of Langerhans cells (LCs), a subset of migratory dendritic cells found in the epidermis, in scrapie transport. They concluded that LCs are not involved in the transport of scrapie PrP^{Sc} from the skin to lymphoid tissues but could have a role in degrading PrP^{Sc} in the skin. The possibility that the transport of PrP^{Sc} is a cell-free process still remains open.

FUNGAL PATHOGENICITY

Role for RNA-binding proteins implicated in pathogenic development of *Ustilago maydis*

Becht, P. *et al. Eukaryot. Cell* **4**, 121–133 (2005)

The basidiomycete *Ustilago maydis* is the causative agent of smut disease in corn crops, particularly sweetcorn. To investigate the function of RNA-binding proteins in *U. maydis*, Becht *et al.* identified 27 open reading frames (ORFs) encoding putative RNA-binding proteins and used PCR to generate gene-replacement mutants in 18 of these ORFs, which represented three of the four main classes of eukaryotic RNA-binding proteins (abbreviated as PUM, KHD and RRM). Extensive phenotypic analysis revealed that most of the ORFs analysed could be replaced without generating a phenotype. However, two RNA-binding proteins that may be involved in *U. maydis* pathogenic development were identified: mutation in Khd4, which belongs to a novel group of KHD proteins, caused pleiotropic effects; and mutation of Rrm4, which has a novel domain organization, resulted in filamentation defects.

EPIDEMIOLOGY

Host immunity and synchronized epidemics of syphilis across the United States

Grassly, N. C. *et al. Nature* **433**, 417–421 (2005)

Syphilis epidemics in the United States oscillate with a periodicity of 10–11 years, and this periodicity has previously been attributed to social and behavioural changes. In a new analysis recently published in *Nature*, Grassly *et al.* compare the incidence of syphilis, to which infected individuals acquire a degree of immunity to re-infection, to the incidence of gonorrhoeae, for which there is no immunity to re-infection, using statistics from 68 US cities over the period 1960–1993. The analysis shows that in fact, rather than being attributable to exogenous factors such as sexual behaviour, the periodic nature of syphilis epidemics is caused by interaction of the *Treponema* spirochaete with the host immune system.