AVIAN INFLUENZA

Solving another piece of the H5N1 puzzle

As the H5N1 avian influenza virus continues to sweep across the globe, its potential to emerge as a human-adapted virus remains high. One crucial determinant of the species specificity of influenza viruses is the haemagglutinin (HA) protein: viruses carrying only 3 of the 16 known avian and mammalian HA subtypes (H1, H2 and H3) have adapted to humans, but in each case, these viruses have caused major pandemics.

Stevens *et al*. now report the high-resolution crystal structure of the HA of a highly pathogenic H5N1 influenza virus (Viet04 HA). The study published in *Science* compares the Viet04 HA structure with an avian H5 HA structure (Sing97) and with HA structures from pandemic influenza A viruses — the deadly 1918 human H1 virus and the 1968 human H3 virus. In addition, the authors use new microarray technology to identify mutations that could allow H5N1 to take hold in the human population.

The general structure of Viet04 HA is similar to other HA structures, consisting of a globular head that contains the receptor-binding domain, a vestigial esterase domain and a membrane-proximal domain. Surprisingly, although the Viet04 HA sequence shows most similarity to that of the avian Sing97 HA, structural comparisons show that Viet04 HA is most closely related to the 1918 H1 HA. The crystal structure of 1918 HA0 revealed two pH-sensitive histidine patches, one in the membrane-proximal domain and one in the vestigial esterase domain, that are thought to promote release of the fusion peptide in the endosome, contributing to pathogenicity. The histidine patch in the membrane-proximal domain is conserved in Viet 04 HA, and additional structural features in the vestigial esterase might contribute further to virulence.

> “…enhanced binding to α2-6 biantennary glycans … is one way in which H5N1 could establish a foothold in the human host.”

Using glycan microarray technology, the authors showed that Viet04 HA binds preferentially to α2-3 sialic-acid glycan receptors, which typifies the binding specificity of avian influenza viruses. Although mutations in the receptor-binding domain that switch H3 HAs from avian to human specificity did not have a similar effect on Viet04 HA, the binding profile of a double mutant, $Gln^{226}Leu$, $Gly^{228}Ser$ Viet04 HA, was markedly altered. This double mutant showed reduced affinity for α2-3 receptors, with significant binding to a natural branched α2-6 biantennary glycan. The respiratory mucins in the human upper airway contain α2-3 glycans, which are thought to filter out avian virus that enters the respiratory tract. Reduced binding to these protective mucins coupled with enhanced binding to α2-6 biantennary glycans in the lower airways is one way in which H5N1 could establish a foothold in the human host.

Shannon Amoils

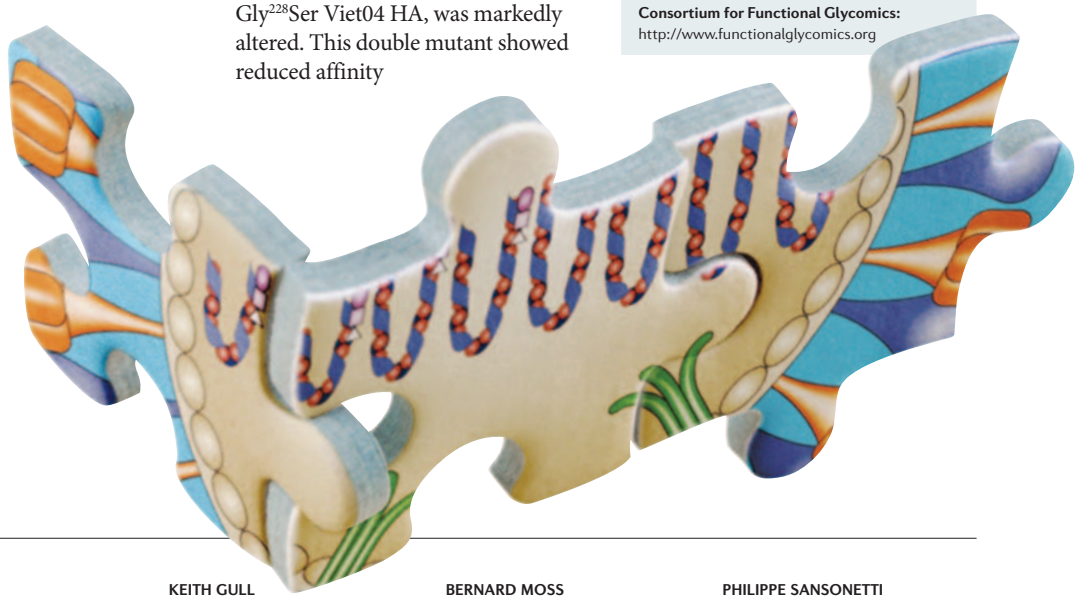
ORIGINAL RESEARCH PAPER Stevens, J. *et al.* Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 16 Mar 2006 (doi:10.1126/science.1124513)

WEBSITES

Ian A. Wilson's laboratory: http://www.scripps.edu/mb/wilson

James Paulson's laboratory: http://www.scripps.edu/mb/paulson

Consortium for Functional Glycomics: http://www.functionalglycomics.org



IN BRIEF

TECHNIQUES AND APPLICATIONS

Use of single-point genome signature tags as a universal tagging method for microbial genome surveys

Van der Lelie, D. *et al. Appl. Environ. Microbiol.* **72**, 2092–2101 (2006)

To obtain meaningful results from genome surveys of microbial communities, novel microbial members must be identified and closely related species discriminated. In this study, van der Lelie *et al.* describe the development of single-point genome signature tags (SP-GSTs), which are identifier tags that are generated from well-defined loci in the genome. An *in silico* evaluation of the SP-GST method revealed that although tags generated to the *rpoC*, *uvrB* and *recA* genes could function as phylogenetic identifiers, their utility is hampered by the dearth of entries in current sequence databases. By contrast, SP-GSTs generated upstream of the 16S rRNA gene contain sufficiently conserved and unique genome sequence to discriminate most organisms from a sample data set to the species level. These upstream 16SRNA SP-GSTs successfully produced tags and correctly identified all members of a defined microbial consortium.

BACTERIAL PHYSIOLOGY

The intestinal life cycle of *Bacillus subtilis* and close relatives

Tam, N. K. M. *et al. J. Bacteriol.* **188**, 2692–2700 (2006)

Although *Bacillus subtilis* is primarily regarded as a soil organism that produces endospores to survive harsh environments, Tam *et al.* provide evidence that *B. subtilis* has a bona fide intestinal life cycle. Mice immunized with *B. subtilis* spores that display a heterologous surface antigen tetanus toxin fragment C (TTFC) did not mount an immune response against the original spore inoculum, but produced antibodies to TTFC displayed on spores that had germinated and resporulated. The expression of *B. subtilis* vegetative- and sporulation-specific genes in the mouse gut provided further proof of intra-intestinal vegetative growth. Natural human faecal isolates of *B. subtilis* persist in the mouse gut for longer periods than laboratory strains, form biofilms and sporulate efficiently in anaerobic conditions. These results indicate that *B. subtilis* proliferates and grows in animal intestine, and the authors suggest that other spore formers might also reveal clandestine intestinal life cycles.

HOST–PATHOGEN INTERACTION

Epithelial cells are sensitive detectors of bacterial pore-forming toxins

Ratner, A. J. *et al. J. Biol. Chem.* 6 Mar 2006 (doi:10.1074/jbc.M511431200)

Many bacteria produce pore-forming toxins (PFTs) that insert into host cell membranes and disrupt their integrity. This study describes a novel mechanism used by epithelial cells to alert the immune response to the presence of PFTs. Ratner *et al.* show that epithelial cells activate the proinflammatory p38 mitogen-activated protein kinase (MAPK) pathway in response to subcytolytic amounts of PFTs. The addition of high-molecular-weight osmolytes to the extracellular medium blocks the pores that are punched in the cell membrane by PFTs and inhibits p38MAPK activation. This implicates epithelial-cell osmosensing mechanisms in proinflammatory responses. Detection of small numbers of pathogens by mechanisms that sense cellular stress could represent an effective early warning system to activate the immune response, and might therefore constitute an as yet unexplored arm of mucosal immunity.

BACTERIAL EVOLUTION

A small leap for adaptation

How does a trait evolve from A to B? Does it take many small steps, or one big one, or does it take one largish step followed by a few small ones? These questions are difficult to answer, mainly because adaptive events are only observed after they have taken place. An experimental evolution study in *Pseudomonas* spp. has captured the first adaptive event as it happens — the fitness advantage of such mutations is high, and so that first step to adaptation is more of a jump.

The view that evolution is a gradual process has been challenged by evidence that large-effect mutations can also underlie adaptive changes. But developing a general rule of adaptation is not easy, especially because beneficial mutations occur rarely. Experimental evolution presents a unique advantage: many beneficial mutations can be recovered, and their adaptive fitness can be compared to that of the ancestral population.

In this study, populations of the bacterium *Pseudomonas fluorescens* were grown in a harsh, carbon-limited medium for approximately 100 generations. The selection coefficients of

ANTIMICROBIALS

A ringing success

In a recent issue of *Science*, Satish Nair, Wilfred van der Donk and colleagues report on an important advance in the understanding of nisin biosynthesis — a finding that could help the search for new antibiotics.

Nisin is a post-translationally modified lantibiotic that is naturally produced by *Lactococcus lactis* and is effective against a wide range of Gram-positive bacteria, including food-borne pathogens such as *Listeria monocytogenes* and *Clostridium botulinum*. It is therefore widely used in the food industry as a preservative. Nisin's microbicidal properties work through several mechanisms: by binding to lipid II — an essential intermediate in peptidoglycan biosynthesis — nisin induces pore formation in the membrane and disrupts cell-wall biosynthesis; in addition, nisin inhibits the outgrowth of bacterial spores.

Nisin contains five thioether rings of varying sizes and conformations that are required for its biological activity. Li *et al.* have now reconstituted the cyclization process of nisin *in vitro*, and report that the cyclase NisC is responsible for the formation of all of the thioether rings. NisA, the precursor peptide, is ribosomally synthesized, followed by dehydration of serine and threonine residues. NisC then catalyses five distinct cyclization reactions to generate five cyclic thioethers: one lanthionine and four methyllanthionines. Once cyclization is complete, NisP removes the leader peptide. Leader-peptide recognition probably has a role in NisC binding, as NisC was unable to convert the dehydrated peptide into its biologically active form after cleavage of the leader peptide.

Li *et al.* also determined the X-ray crystal structure of NisC, which revealed unexpected similarities in fold and substrate activation with mammalian

the 68 fixed beneficial mutations that were recovered followed a bell-curve distribution; they also occurred at a higher rate than expected (3.8×10^{-8} per cell division) and had a much greater selective advantage (2.1).

The fact that the first beneficial steps in evolution are larger than previously supposed has theoretical importance, but it also has a direct bearing on modelling the evolutionary trajectory of microbes that are harmful to human health.

Tanita Casci, Senior Editor,
Nature Reviews Genetics

ORIGINAL RESEARCH PAPER Barrett, R. D. H. *et al.* Mutations of intermediate effect are responsible for adaptation in evolving *Pseudomonas fluorescens* populations. *Biol. Lett.* 14 February 2006 (doi:10.1098/rsbl.2006.0439)
FURTHER READING Kassen, R. & Bataillon, T. Distribution of fitness effects among beneficial mutations before selection in experimental populations of bacteria. *Nature Genet.* **38**, 484–488 (2006) | Orr, H. A. The genetic theory of adaptation: a brief history. *Nature Rev. Genet.* **6**, 119–127 (2005)

farnesyl transferases. Similar to farnesyl transferase, a single zinc ion is situated at the top face of the double α-barrel in NisC. These structural similarities suggest that human homologues of NisC might post-translationally modify a cysteine of a protein substrate.

Although nisin has been in use as a food preservative worldwide for decades, it has not induced the widespread biological resistance encountered by many antibiotics. This is thought to be due to its multiple modes of action and the nature of the lipid II target, which is not a protein and is not easy to modify by the bacteria. The newfound knowledge of how nisin is biosynthesized, and the enzymes involved in this process, could be useful in the search for novel antibiotics that could avoid the development of antibiotic-resistant bacteria.

Annie Tremp

ORIGINAL RESEARCH PAPER Li, B. *et al.* Structure and mechanism of the lantibiotic cyclase involved in nisin biosynthesis. *Science* **311**, 1463–1467 (2006)
FURTHER READING Christianson, D. W. Five golden rings. *Science* **311**, 1383 (2006) | Cotter, P. D., Hill, C. & Ross, R. P. Bacteriocins: developing innate immunity for food. *Nature Rev. Microbiol.* **3**, 777–788 (2005)

Germinating spores of the fungus *Magnaporthe grisea*, which causes rice blast disease. Image courtesy of M. Gilbert, University of Exeter, UK.

FUNGAL PATHOGENESIS

Understanding rice blast disease

One of the mechanisms by which plant pathogens cause disease is the delivery of proteins directly into plant cells, where these proteins can promote plant-tissue colonization and suppress plant defences. Mechanisms that bacterial phytopathogens use to effect this delivery are known, but it has been unclear how fungal phytopathogens do so. Now, research published in *Nature* shows that the P-type ATPase Apt2 is required for tissue colonization and for efficient delivery of virulence-associated proteins by the fungus *Magnaporthe grisea*, the causative agent of rice blast disease.

The *M. grisea* genome encodes many secreted proteins, which could be involved in suppression of host defence, so Talbot and colleagues set out to study the mechanisms by which these proteins are secreted. The *M. grisea* genome was found to contain four genes that putatively encode aminophospholipid translocases (APTs), enzymes that are involved in membrane-fusion events during intracellular protein trafficking. One of these — *APT2* — is closely related to the *DRS2* gene family of APTs in the yeast *Saccharomyces cerevisiae*, and members of this family are necessary for efficient Golgi function and are involved in the endocytic and exocytic trafficking pathways. Complementation of yeast mutants with *APT2* confirmed that Apt2 fulfils some of the functions of a Drs2-family protein. Furthermore, deletion of *APT2* significantly impaired the ability of *M. grisea* to cause rice blast disease, owing to reduced infection of plant roots and leaves. This was shown to be associated with a reduction in exocytosis, because *APT2* deletion mutants accumulated abnormal membrane-bound structures in the cytoplasm and could not secrete various enzymes. In addition, inoculation of plant leaves with these mutants elicited reduced plant-defence responses, indicating that Apt2 might be required for the secretion of proteins that are recognized by the host during a resistance response.

Identification of Apt2 as a fungal factor that is required for exocytosis during plant-tissue colonization contributes to our understanding of fungal pathogenicity. It also has implications for the control of rice blast disease, because proteins that are involved in infection could be targets for the development of new fungicides.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Gilbert, M. J., Thornton, C. R., Wakley, G. E. & Talbot, N. J. A P-type ATPase required for rice blast disease and induction of host resistance. *Nature* **440**, 535–539 (2006)

BACTERIAL PATHOGENESIS

Anthrax researchers complete triumvirate

Bacillus anthracis produces two bipartite toxins, which have different cellular effects. Both contain the same receptor-binding moiety, protective antigen (PA), and this is combined with different effector moieties — PA in combination with oedema factor (EF) forms oedema toxin (ET), an adenylate cyclase that increases the intracellular concentration of cAMP, causing oedema, whereas PA in combination with lethal factor (LF) forms lethal toxin (LT), a metalloproteinase that cleaves mitogen-activated protein kinase kinases, disrupting intracellular signalling.

The mechanism of entry of the toxins into host target cells is relatively well understood, with the exception of one missing element. Two cell-surface proteins have been confirmed as receptors for PA, the anthrax toxin receptor (ATR) and capillary morphogenesis protein 2 (CMG2), but it has long been suspected that a third cell-surface protein might be involved in internalization of the toxin–receptor complex, as PA binding to ATR or CMG2 alone is not sufficient for toxin entry. The third protein has now been identified by Wensheng Wei and Quan Lu in Stanley Cohen's lab, in collaboration with G. Jilani Chaudry and Stephen Leppla, and is reported in a recent issue of *Cell*.

The authors set out to try and identify cellular genes involved in LT/ET toxicity using a phenotypic screen in which a lentivirus vector was used to introduce antisense RNAs against chromosomal genes into a human cell line, and clones deficient in PA internalization could be selected. Screening of the selected clones led to the identification of the gene encoding low-density lipoprotein receptor-related protein 6 (LRP6), a cell-surface protein known to be a co-receptor for Wnt signalling.

The observation that murine macrophages transfected with a lentivirus vector expressing *lrp6*-specific short interfering RNAs showed improved survival when challenged with native LT compared with cells transfected with control vectors, coupled with western blot and deconvolution microscopy analyses estimating the abundance of cell-surface and internalized PA in the absence of LRP6, suggested that LRP6 might be involved in PA internalization. This was confirmed in further work using a dominant-negative LRP6 mutant protein lacking most of the cytoplasmic domain, which also proved not only

INNATE IMMUNITY

CRIg clears out the bad guys

Pathogens in the circulation that become coated with fragments of complement component 3 (C3) — a process known as C3 opsonization — are phagocytosed by Kupffer cells — liver-resident macrophages — and are thereby cleared from the host. A report recently published in *Cell* has now identified a new complement receptor, complement receptor of the immunoglobulin superfamily (CRIg; also known as Z39Ig), as the receptor that is required for this efficient phagocytosis of C3-opsonized pathogens by Kupffer cells.

Previous studies indicated that although Kupffer cells express one of the receptors for fragments of C3, complement receptor 3, this receptor does not seem to have a role in the clearance of C3-opsonized pathogens. So, when Helmey *et al.* found that both human CRIg and mouse CRIg were expressed by Kupffer cells and subsets of macrophages resident in other tissues, they set out to determine whether CRIg was the receptor through which Kupffer cells mediate phagocytosis of C3-opsonized pathogens.

There are two alternative splice variants of human CRIg, a long form and a short form, but only a single form of mouse CRIg, which has 67% sequence homology with the short isoform of human CRIg. All three CRIg proteins were shown to bind soluble forms of the C3 fragments C3b and iC3b, as well as particles opsonized with C3b and iC3b. The *in vivo* function of CRIg was analysed using mice lacking expression of CRIg. Although these mice had normal numbers of Kupffer cells and other populations of tissue-resident macrophages, CRIg-deficient Kupffer cells did not bind soluble C3b and iC3b and, compared with wild-type Kupffer cells, had impaired binding to C3-opsonized IgM-coated sheep erythrocytes. Furthermore, Kupffer cells in CRIg-deficient mice infected intravenously with either *Listeria monocytogenes* or *Staphylococcus aureus* internalized fewer bacteria than Kupffer cells in similarly infected wild-type mice. In addition, more live bacteria were recovered from the blood, spleen and tissues of infected CRIg-deficient mice. This defect in clearance of pathogens from the circulation resulted in increased levels of serum cytokines in CRIg-deficient mice infected intravenously with *L. monocytogenes* and increased mortality.

Mechanistically, it was shown that CRIg on the surface of Kupffer cells

that LRP6 is necessary for toxin lethality and not just internalization, but also that the intracellular domain of LRP6 is required for both functions. To establish whether LRP6 interacts with either ATR or CMG2 and if so, what the functional significance of this interaction is, co-immunoprecipitation and fluorescence microscopy were used, and it was found that LRP6 interacts with both ATR and CMG2, and the authors propose a model in which this interaction provides the signal for internalization of the toxin–receptor complex.

The paper concludes with data showing that anti-LPR6 antibodies can protect a macrophage cell line from the lethal effects of LT, suggesting that this work could have ramifications for the treatment of the later stages of anthrax disease.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Wei, W. *et al.* The LDL receptor-related protein LRP6 mediates internalization and lethality of anthrax toxin. *Cell* **124**, 1141–1154 (2006)

is constitutively being internalized and then recycled back to the cell surface. Consistent with this finding, internalized CRIg was observed to co-localize with transferrin, which is a marker of recycling endosomes, and not with lysosomal-associated membrane protein 1, which is a marker of lysosomes. Further analysis showed that during phagocytosis of C3-opsonized particles, CRIg is recruited to the forming phagosome but returns to the recycling endosomes before, or during, phagosome–lysosome fusion.

This study identifies CRIg as the main receptor expressed by Kupffer cells for pathogens opsonized with C3 fragments that induces phagocytosis, and thereby clearance, of these pathogens. As such, CRIg functions as a key mediator of the first line of host defence against systemic infections.

Karen Honey, Senior Editor,
Nature Reviews Immunology

ORIGINAL RESEARCH PAPER Helmey, K. Y. *et al.* CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* **124**, 915–927 (2006)

BACTERIAL PHYSIOLOGY

The Mtb proteasome: an open and shut case

Prokaryotic proteasomes are a rare breed: only the Archaea and a few members of the Actinomycetes have been shown to possess a proteasome. But now, this exclusive club has a new member — two groups report the detailed biochemistry and structure of the *Mycobacterium tuberculosis* (Mtb) 20S proteasome.

Genes that encode putative proteasome components (such as the *prcBA* operon) had been identified in Mtb, but the presence of a proteasome in this bacterium remained unproven. A team led by Carl Nathan co-expressed recombinant PrcA and PrcB in *Escherichia coli* and showed that these proteins assemble into a 750-kDa, 28-subunit cylindrical structure with catalytic activity and the inhibitor profile of a proteasome, proving that Mtb does possess an authentic 20S proteasome.

In a parallel collaborative study, Guiqing Hu in Huilin Li's lab and collaborators solved the crystal structure of the Mtb proteasome at 3.0-Å resolution and revealed that its overall architecture is similar to that of archaeal and eukaryotic proteasomal systems. The Mtb proteasome cylinder (~150 Å in height with a diameter of ~115 Å) is made up of two outer α-subunit heptameric rings and two inner β-subunit heptameric rings. The peptides that might obstruct substrate entrances at the two extreme ends of the cylinder are not visualized, owing to partial disorder of the α-subunit N termini. By contrast, the ends of eukaryotic proteasomes are seen to be tightly closed, and substrates enter into the catalytic cylinder only when these ends open in response to the binding of regulatory molecules.

Gang Lin in Nathan's lab and collaborators found that the catalytic activity of the Mtb proteasome was nearly 200-fold less than that of the actinomycete *Rhodococcus*, which suggested that substrates might be excluded from the catalytic chamber and that, similar to eukaryotes, the Mtb proteasome might be 'gated'.

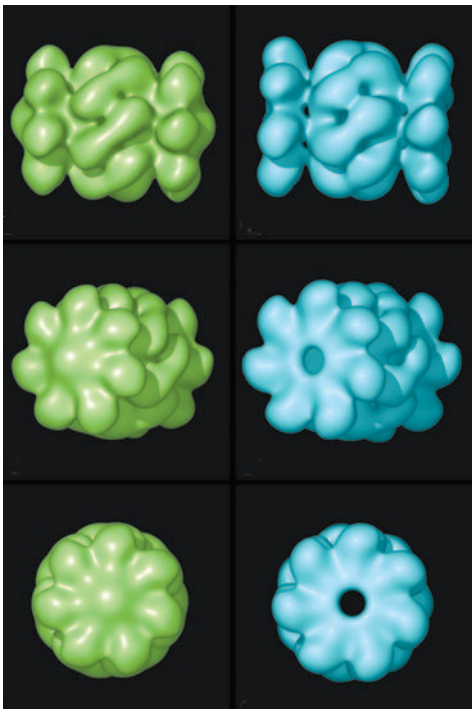
Negative staining and cryo-electron microscopy by both groups confirmed that the central chamber of the proteasome cylinder was indeed closed to the exterior. When Lin and colleagues deleted eight amino-acid residues from the N terminus of the α-subunit, the cylinder of the mutant proteasome was open at both ends, identifying the N-terminal octapeptides of the α-subunits as crucial components of the proteasomal gate.

The peptidolytic activity of the open-ended mutant was surprisingly broad. Whereas other prokaryotic proteasomes cleave only hydrophobic peptides, the Mtb open-ended proteasome cleaved oligopeptides after hydrophobic, basic, acidic and small neutral amino acids. Analysis of the topology of the catalytically active β-subunits in the substrate-binding pocket by Li and his team provided an explanation for this promiscuous peptidolytic activity. The proteasomes of all prokaryotes studied so far have only one type of β-subunit. But the substrate-binding pocket of Mtb is chimeric: its upper surface has features that resemble the eukaryotic β5-subunit and its lower surface is similar to eukaryotic β1- and β2-subunits, which confers the broad substrate specificity that is reminiscent of eukaryotic systems.

Finally, Li and co-workers determined the binding mode and inhibition mechanism of a proteasomal inhibitor that is an analogue of a drug used to treat the blood disorder myeloma. As the Mtb proteasome has a crucial role in mycobacterial defence to host nitrosative stress, these elegant and comprehensive studies might well be the starting point for the design of proteasome-targeted anti-tuberculosis therapy.

Shannon Amoils

ORIGINAL RESEARCH PAPERS Lin, G. *et al.* *Mycobacterium tuberculosis prcBA* genes encode a gated proteasome with broad oligopeptide specificity. *Mol. Microbiol.* **59**, 1405–1416 (2006) | Hu, G. *et al.* Structure of the *Mycobacterium tuberculosis* proteasome and mechanism of inhibition by a peptidyl boronate. *Mol. Microbiol.* **59**, 1417–1428 (2006)



Structure of the wild-type Mtb proteasome (in side, oblique and top views, left panel) and an α-subunit N-terminal deletion mutant (also in side, oblique and top views, right panel) derived by EM and single-particle image reconstruction. The substrate entrance is blocked in the wild-type structure but open in the deletion mutant, suggesting that the Mtb proteasome is gated. Image courtesy of Huilin Li, Brookhaven National Laboratory, Upton, New York, USA.