News in brief

Viral Targets and Mechanisms

Filling in the pocket: a new strategy against Dengue?

Researchers at the Children's Hospital and Harvard Medical School, Boston (http://web1.tch.harvard.edu/) have identified a unique pocket on the surface of the Dengue virus that is involved in host-cell binding and fusion [1]. The pocket is located near a pivotal hinge region, which helps the Dengue virus to change its structural conformation, thus enabling it to fuse with host cells. Inserting compounds into this pocket could block the fusion process and prevent the virus from infecting cells.

Stephen Harrison, leader of the research group, commented: 'Inhibiting fusion is a sensible way of inhibiting viral replication'. Further structural analysis of this pocket could pave the way for developing novel chemotherapeutics against Dengue hemorrhagic fever, for which there is no treatment or vaccine currently available.

When an infected mosquito injects the Dengue virus into humans, the virus attaches and enters the host cell via the major envelope glycoprotein E; this protein punches holes onto the membrane of host cells, permitting the virus to unload its genes inside the cell and initiate viral replication. Detailed structural studies of the E protein revealed a hydrophobic ligand-binding pocket, near the hinge region, that has a significant role in changing the conformation of the virus. A tiny flap in the E protein usually hides this ligand-binding pocket; however, when the pocket is filled with a hydrophobic ligand, the flap opens and acts as a piece of scaffolding, keeping the protein propped open at the hinge.

Yorgo Modis, the first author of the paper, adds '…knowing the size and shape of the pocket should advance the search for a drug'. Hence, the research team is now investigating the structure of the E protein and how it changes its shape to enable the virus to infect host cells. Results from these studies should also help drug discovery and development in other diseases caused by viruses with similar envelope proteins.

The WHO (http://www.who.org) estimates that there are 50 million cases of Dengue infection and 24,000 deaths per year, worldwide, most of which occur in the tropics. However, recent cases in the USA have led the US Centers for Disease Control (http://www.cdc.gov) to describe Dengue as one of the most important mosquito-borne viral disease affecting humans.

1 Modis, Y. *et al.* (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0832193100 (http://www.pnas.org)

How HTLV-1 hacks in

Human T-lymphotropic virus (HTLV-1) can cause devastating illnesses, including adult T-cell lymphoma and diseases of the nervous system. Scientists looking at how HTLV-1 infects cells have now shown that one of its proteins, p12^{I}, contains a motif found in host-cell regulatory proteins. Their findings indicate that HTLV-1 uses this motif to hack into normal cell processes, providing crucial new insight into how this retrovirus attacks and how it might be stopped [2].

In early stages of HTLV-1 infection, the protein p12^{I} promotes inappropriate activation of T lymphocytes. By speeding up host-cell division, the retrovirus facilitates its own replication. Researchers at Ohio State University (http://www.osu.edu) have now identified a motif within p12^{I} that is highly homologous to sequences that mediate binding of host-cell proteins to the enzyme calcineurin. Seung-jae Kim and colleagues demonstrated that this motif also enables p12^{I} to bind to calcineurin, the first report of calcineurin-binding activity in a retroviral protein.

They went on to show that binding of calcineurin by p12^{I} blocks binding of the enzyme by a crucial host protein containing the same motif, nuclear factor of activated T cells (NFAT). Because the interaction of NFAT with calcineurin normally activates cell division, it seems that p12^{I} uses its calcineurin-binding motif as a password, to mimic NFAT and to trigger inappropriate replication.

These important results suggest a novel mechanism for retrovirus-mediated cell activation. Michael D. Lairmore, leader of study, said: 'This discovery may help us explain just how HTLV-1 turns these cells on, leading to replication'.

2 Kim, S-J. *et al.* (2003) A conserved calcineurin-binding motif in human T lymphotropic virus type 1 p12^{I} functions to modulate nuclear factor of activated T cell activation. *J. Biol. Chem.* 278, 15550–15557

Z-DNA unravelled? Viral and anti-viral clues

Important findings indicating the function of *Z*-DNA in cellular defences against viral attack have been released by researchers at Arizona State University (http://www.asu.edu) and Massachusetts Institute of Technology (http://www.mit.edu). The research has provided the bonus by-product of potential development of new anti-viral drugs that could be effective against viruses such as smallpox [3].

Z-DNA was originally discovered by Alexander Rich, one of the paper's corresponding authors. This 'flip'-form of the normal *B*-DNA conformation has long been an enigma. To solve the mystery, the research team used a crucial vaccinia virus protein, E3L, necessary for the virus to disable animal cell defences. The N-terminal domain active site of the E3L protein was shown to work by binding to *Z*-DNA and interfering in its operation. On replacing the active site of E3L's with a *Z*-DNA binding domain from another protein, ADAR1, the vaccinia virus retained lethality. Further studies revealed that mutations affecting the *Z*-DNA binding ability diminished the lethality of the modified virus. Conversely, such modified non-lethal forms of the virus can be made lethal by mutating the active site protein back to a *Z*-DNA binding form.

This experimental evidence leads to a real possibility that *Z*-DNA has a role in regulating the transcription of specific

anti-viral genes. The next step will be to find out what genes are induced when infecting an animal cell with a wild-type virus, as opposed to infecting with a virus without a *Z*-DNA binding protein.

The crystal structures of *Z*-DNA binding domains on viral proteins are mostly solved. Hence, a molecule could be designed to fit into the binding site on the viral protein and keep it from binding *Z*-DNA, thus preventing viruses such as smallpox, which is similar to the vaccinia virus, from causing the disease.

3 Kim, Y.G. *et al*. (2003) A role for Z-DNA binding in vaccinia pathogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas. 0431131100 (http://www.pnas.org)

Cancer Targets and Mechanisms

Raising melanoma protection by a power of PTEN

New research has uncovered one of the mechanisms that trigger melanomas and points to a potential means of treating this cancer [4].

Malignant melanoma is the most deadly form of skin cancer and one of the most increasingly common forms of cancer in general. Melanomas are triggered in about 90% of severe sunburn cases, but the immune system is often able to eliminate them. Sometimes, however, the cancerous cells are able to adapt and become malignant. Exactly how this occurs is poorly understood.

A new study from Penn State College of Medicine (http://www.hmc.psu.edu/college/) has identified one of the key mechanisms that triggers malignancy. The team, led by Gavin Robertson, have suggested the importance of PTEN in regulating melanoma aggression.

The PTEN gene has previously been identified as a tumour-suppressor gene and has been implicated in several other forms of cancer. In normal cells, PTEN functions in the apoptosis pathway, ensuring that malfunctioning cells are destroyed. When the gene is absent or dysfunctional, such cells can thrive and become malignant.

In this new study, the effects of the PTEN gene on melanoma growth were studied in mice. Chromosome 10, which contains the PTEN gene, was transferred from healthy cells into melanoma cells. Introduction of the gene switched off the tumour cell growth, enabling apoptosis to resume. However, the effect was only temporary, with the cells eventually acquiring resistance to the treatment. This is the first time that loss of PTEN has been shown to promote the growth of malignant melanoma.

'These discoveries may lead to another crucial weapon in the rather small arsenal of treatments available for this dangerous disease and offer the first hope for a new melanoma target in decades,' commented Robertson. One such therapy could involve introduction of the PTEN gene into melanoma cells via a harmless viral carrier targeted to the cells.

4 Stahl, J.M. *et al.* (2003) Loss of PTEN promotes tumour development in malignant melanoma. *Cancer Res.* 63, 2881–2890

Scientists identify mechanism for tumour death by radiation

Ionizing radiation is used widely to treat patients with solid tumours, and for many years it was thought to kill cancer cells solely by damaging their DNA. However, a recent study by Monica Garcia-Barros *et al.*, based at Memorial Sloan-Kettering Cancer Center (http://www.mskcc.org), has provided the first genetic evidence that damage to the blood vessels feeding tumours also has a primary role in tumour regression [5].

The blood-vessel endothelial cells, recruited during angiogenesis, are targeted by radiation to die – not by DNA-damage-induced cell death, however, but via a specialized form of apoptosis.

Researchers studied how endothelial cells within tumours respond to radiation, using a mouse model deficient in acid spingomyelinase (asmase), an enzyme needed for endothelial cells to undergo apoptosis. Melanoma and fibrosarcoma cells were implanted into normal (asmase$^{+/+}$) and spingomyelinase-deficient (asmase$^{-/-}$) mice and the level of endothelial apoptosis was measured in the resultant tumours. The asmase$^{-/-}$ mice showed reduced endothelial apoptosis, a tumour growth rate double that of normal mice, and were also resistant to radiation-induced tumour regression (at clinically relevant doses). 'Our study confirmed that acid spingomyelinase affects the endothelium and that in turn plays a role in a tumour's growth and its response to radiation,' said Garcia-Barros.

The sensitivity of asmase$^{+/+}$ endothelial cells to apoptosis is consistent with the cells synthesizing 20-times more asmase than other cells. This large excess could have a role in tissue remodelling and wound repair, processes that involve significant levels of neoangiogenesis and endothelial apoptosis. This suggests that a normal function of endothelial asmase is to delimit the rapid growth associated with tumour development.

Further research is needed – into radiation levels, timing of therapy and, possibly, combining radiation and anti-angiogenic therapies – for a better understanding of this novel form of radiation-induced cell death, which could ultimately lead to new, improved strategies for cancer treatment.

5 Garcia-Barros *et al.* (2003) Tumour response to radiotherapy regulated by endothelial cell apoptosis. *Science* 300, 1155–1159

Miscellaneous

Liquid assets control drug delivery

Artificial pancreases and liquid computers are just two of the potential applications of a new microfluidics technology [6]. For the first time, microfluidic memory and control devices with no moving parts or electronics have been created by harnessing the special properties of an aqueous polymer solution.

Microfluidics applications are receiving increased attention in the field of drug delivery. A device without electronic systems that can release drugs in a controlled way would be ideal for use inside the body. However, a large barrier to such applications remains: how can these devices incorporate memory and control systems without resorting to electronics? In an ingenious development from scientists at the University of California, San Diego (http://www.ucsd.edu/) and the California Institute of Technology (http://www.caltech.edu/), both a memory and a control system have been fabricated with no moving parts or electronics.

Genomics and Proteomics

Cracking the anthrax code

The use of anthrax as a weapon means that developing vaccines and treatments for the disease have become important goals. Recent publication of the first complete genome sequence of anthrax-causing bacteria, and comparison of this sequence with those from related bacteria that do not cause the disease, are significant steps forward for anthrax research [7,8].

Unlike its close relatives, *Bacillus anthracis* can cause lethal inhalation anthrax. Previous work has shown that its characteristic virulence and toxicity are encoded by genes on its two plasmids – circles of DNA that are separate from the main chromosome. In a study coordinated by scientists at the Institute for Genomic Research (http://www.tigr.org/), Tim Read and colleagues looked more closely at the whole *B. anthracis* genome [7]. They sequenced the DNA of the particularly virulent Ames strain, which in recent years has been used in terror attacks. The team found that the non-plasmid part of the genome, the chromosome, also carries genes that might contribute to the pathogenicity of anthrax. In addition, they were able to identify potential targets for vaccines and drugs.

Read and colleagues went on to compare the *B. anthracis* genome sequence with that of *B. cereus*. Although the plasmid DNA varied considerably, the chromosomal DNA sequences were almost identical. Surprisingly, most of the putative chromosomal virulence genes from *B. anthracis* had *B. cereus* homologues, a finding supported by an independent study led by scientists based at Integrated Genomics in Illinois (http://www.integratedgenomics.com/) [8].

Read and *et al*. suggest that *B. anthracis* might be so different from its less dangerous relatives because the common chromosomal genes have distinct functions or expression levels, possibly because of the plasmid genes.

'Deciphering the anthrax genome is important to a wide range of biochemical and biodefense research,' says Claire M. Fraser, supervisor of the *B. anthracis* genome project. 'The genome sequence will benefit research projects to find targets for new drugs and vaccines as well as to improve anthrax detection and diagnosis.'

7 Read, T.R. *et al*. (2003) The genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria. *Nature* 423, 81–86

8 Ivanova, N. *et al.* (2003) Genome sequence of *Bacillus cereus* and comparative analysis with *Bacillus anthracis*. *Nature* 423, 87–91

Genome complexity reaches new heights

Scientists at the University of North Carolina at Chapel Hill (UNC; http://www.unc.edu) have serendipitously determined differences in the physical properties of yeast chromatin, the 'gatekeeper' of DNA packaging [9]. This newly discovered level of genomic complexity and organization involving chromatin variation could exist in all eukaryotic cells.

DNA packaging into chromatin not only serves to compact genetic material but also determines which genes are turned on or off by controlling the accessibility of genome parts to regulatory proteins. Defects in the histone proteins that organize DNA can lead to embryonic development defects due to their influence on underlying gene activity.

The team at UNC were investigating the global distribution of regulatory influence of a particular histone in yeast. The yeast was fixed with formaldehyde, but the later step of crosslinking reversal using heat was inadvertently omitted. Based on differential crosslinking efficiency in different genetic regions, the yeast chromatin could be fractionated into two functionally distinct parts: one containing RNA polymerase II transcribed sequences; and one comprising noncoding sequences and genes transcribed by RNA polymerases I and III.

These results reveal a genome-wide molecular mechanism for marking sequences that reflect their assigned role as either genes or 'junk DNA' in yeast, and might be applicable to other organisms. Thus, this tool carries the potential for characterization of changes in chromatin structure that accompany different genetic, environmental and disease states.

9 Nagy, P.L. *et al.* (2003) Genomewide demarcation of RNA polymerase II transcription units revealed by physical fractionation of chromatin. *Proc. Natl. Acad. Sci. U. S. A.* May 15/epub ahead of print (http://www.pnas.org)

The flow control device, called a flux stabilizer, consists of a series of identical U-shaped chambers, each of which has uniform thickness (100 μm) throughout, except for a narrow outlet at one end. The U-bends, which are made from silicon rubber, line up head to tail, with the narrow outlet leading into the larger tail end of the next chamber; this leads to what, from a more macroscopic viewpoint, looks like a long, wavy tube. When a polymer fluid is passed through the device, the series of bottlenecks and chambers forces it to expand and contract. Despite changing pressure, the polymer's viscoelastic properties enable it to flow at a constant rate, a key requirement for the controlled delivery of drugs.

The second device is analogous to a digital flip-flop memory. The researchers were able to construct a fluidic chamber that can maintain a stable 'high' or 'low' state, which are comparable to the ones and zeros of an electronic system. Again, the device incorporates wide chambers and narrow bottlenecks to control polymer flow. After feeding the polymer through the first bottleneck into a 'crossroads', it can exit through one of two outlets, which represent two stable and mutually exclusive states. Egress through one of these exits 'locks' the polymer to that outlet until a large pressure change causes the polymer to flow through the second outlet.

The authors are enthusiastic about the application of their devices to drug delivery. 'One option for this research would be an artificial pancreas for patients with diabetes,' commented Stephen Quake, an author from CalTech. 'The device could measure glucose levels and dispense appropriate amounts of insulin.'

6 Groisman, A. *et al.* (2003) Microfluidic memory and control devices. *Science* 300, 955–958

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