that such a drug is effective: 'the enzyme just can't work.' In addition, a compound that binds at a protein's active site is less likely to lead an organism to develop resistance. Explains DeLucas, amino acids on the surface of a protein could be easily mutated without affecting function but the active site is different. 'If you look at any protein…there will be certain amino acids that are critical for it to function.' By making a drug that binds to those specific residues, you use an area of the protein that is less likely to naturally mutate and thereby confer resistance.

New antimicrobial drugs

J. Todd Weber, of the National Center for Infectious Disease at the US Center for Disease Control (http://www.cdc.gov), says that it is 'clear that we do need new classes of antimicrobial drugs,' because the 'rates of resistance are increasing' all the time. Although Weber is not familiar with the unpublished details of the current work, he noted, 'any advance to create new drugs that get around the mechanisms of developing antibiotic resistance would certainly be valuable'.

Of course, today we must also consider a non-natural source of mutations that might lead to drug resistance: those engineered by potential bioterrorists. DeLucas suggests that by making the active site the drug target, you could protect the drug's efficacy. Vaccines, too, are subject to lose their effectiveness through natural protein mutations. 'Does this mean we should not use vaccines' to protect people against a biological attack, DeLucas asks. 'No. They present another hurdle for terrorists,' and fighting biological warfare, he says, 'should use a dual-pronged approach'. DeLucas is betting that one day the newly developed compounds will be used in that fight.

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News in brief

Targets and mechanisms

The worm has turned

Scientists at Massachusetts General Hospital (http://www.mgh.harvard.edu/) and their colleagues have scoured thousands of genes in the *Caenorhabditis elegans* worm and have identified hundreds of promising candidates that could determine how fat is stored and used in a variety of animals [1]. Their findings represent the first survey of an entire genome for all genes that regulate fat storage.

The research team, led by Gary Ruvkun, used RNA-mediated interference (RNAi) to disrupt the expression of each of the 16,757 genes of *C. elegans* in a systematic screen of the genome for genes that are necessary for normal fat storage. In this way they identified ~300 genes that, when inactivated, cause reduced body fat and ~100 genes that cause increased fat storage when turned off. The identified genes were diverse and included both the expected genes involved in fat and cholesterol metabolism, as well as new unexpected candidates. Many of the fat regulatory genes identified in this study have counterparts in humans and other mammals.

'This study is a major step in pinpointing fat regulators in the human genome,' says Ruvkun. 'Of the estimated 30,000 human genes, our study highlights about 100 genes as likely to play key roles in regulation of fat levels,' he continued. Most of these human genes had not previously been predicted to regulate fat storage and could pave the way for designing drugs to treat obesity and its associated diseases such as diabetes.

1 Ashrafi, K. *et al.* (2003) Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421, 268–272

Piecing together the HIV virus

New information has been revealed on the interactions that mediate virus assembly. A team led by Peter Prevelige of the University of Alabama at Birmingham (UAB; http://main.uab.edu/) used MS to probe the assembly of HIV-1 capsid protein [2], a target with obvious therapeutic significance.

Much data has been collected over the past few decades on the structures of the protein constituents of viruses. However, the means by which the numerous capsid subunits unite to form the virus particle are less-well understood, especially in retroviruses. New research on the nature of such interactions has obvious implications for drug research. Advanced structural knowledge would be an important foundation for developing anti-viral drugs that could target interactions between capsid particles and disrupt the virus.

The team from UAB used high-resolution MS to probe the interactions between individual HIV-1 capsid proteins. The HIV-1 virus, like other retroviruses, has frustrated attempts at detailed structural

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characterization. It is non-icosahedral, enveloped and can take several forms, so traditional techniques such as X-ray crystallography have been ineffective. By using MS, however, the team were able to deduce which parts of the protein were involved in interactions with other capsid proteins. Comparing amide hydrogen atoms on a soluble capsid protein with those in a supramolecular complex, they measured the relative ease of deuterium substitution. In areas where protein–protein interactions form during assembly, the exchange will be limited, providing evidence of amino acid crosslinking. The resulting interaction map builds on existing evidence to provide the most detailed picture yet of HIV-1 formation.

The research will hopefully open the door to new HIV therapies, which are urgently needed; as Prevelige notes, 'The problem is that HIV drugs tend to become less effective over time. New drugs aimed at new targets would provide additional treatment options.'

2 Lanman, J. *et al.* (2003) Identification of novel interactions in HIV-1 capsid protein assembly by high-resolution mass spectrometry. *J. Mol. Biol.* 325, 759–772

blue cheese holds promise for Alzheimer's research

Many human neurodegenerative disorders are characterized by the accumulation of insoluble ubiquitin-containing aggregates in the CNS. A study reporting the generation of mutant flies with such aggregates, and the identification and characterization of the gene responsible, sheds new light onto the causes of human neurodegenerative diseases [3].

A team led by Kim D. Finley of the Salk Institute for Biological studies (http://www.salk.edu/) and Michael McKeown of Brown University (http://www.brown.edu/) has generated mutant flies with features that are often used to diagnose human neurodegenerative disorders. The adult mutant flies die prematurely, their brains riddled with dark deposits and showing extensive neuronal cell death. The deposits were found to contain insoluble aggregates of ubiquitinated proteins and amyloid-precursor-like protein – the *Drosophila* version of amyloid-precursor protein, which forms plaques in human Alzheimer's disease.

Finley and colleagues went on to identify and characterize the mutated gene, which they called *blue cheese* (*bch*) because the dark brain deposits reminded them of the mould in veined cheeses. They realized that the blue cheese protein, BCH, is highly conserved in animals ranging from nematode worms to humans. They also showed that it contains motifs implicated in protein processing and vesicular transport.

'These observations alone suggest a high likelihood that alterations in human blue cheese will contribute to some degenerative disorders in humans', said Finley. But, as Finley went on to explain, this is not the only evidence implicating the human gene. 'Analysis of the human genetic map shows that *blue cheese* gene is in a region associated with several familial neurodegenerative diseases.' Further work on the *bch*-mutant flies holds great promise for those seeking to understand the molecular basis of Alzheimer's disease and other human neurodegenerative disorders.

3 Finley, K.D. *et al.* (2003) *blue cheese* mutations define a novel, conserved gene involved in progressive neural degeneration. *J. Neurosci.* 23, 1254–1264

How to catch leukaemia

Approximately 10–20 million people worldwide are infected with the human T-lymphotropic virus type 1 (HTLV-1). Most infections are asymptomatic, but in 2–3% of people the virus causes adult T-cell leukaemia.

Until recently it was not known how the virus spreads, but a collaboration between researchers in the UK and Japan, directed by Professor Charles Bangham from Imperial College London (http://www.ic.ac.uk) suggests that HTLV-1 spreads by subverting normal T-cell behaviour [4].

Normally, viruses are spread by the release of thousands of virus particles from a single infected cell. HTLV-1, however, does not infect other cells by the release of particles, but by cell–cell contact.

Using confocal microscopy, in combination with fluorescence imaging techniques, the researchers were able to examine *in vitro* the distribution of the HTLV-1 genome and encoded proteins in blood cells from infected individuals. They noticed that cell–cell contact rapidly induces polarization of the cytoskeleton of the infected cell to the cell–cell junction. HTLV-1 core protein complexes and the associated HTLV-1 genome then accumulate at the cell–cell junction before being transferred into the uninfected cell.

'Although this research is not a cure,' comments Bangham, 'it does show how the virus is able to spread through the body and infect other people. From this we hope to be able to develop more effective treatments for this fatal disease'. Indeed, although HTLV-1 appears to be reliant on this route of infection, it remains to be seen whether other lymphotropic viruses, such as HIV-1, also have the ability to spread infection in this way.

4 Igakura, T. *et al.* (2003) Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* 10.1126/science.1080115 (epub ahead of print; http://www.sciencemag.org)

Microsatellites identify leprosy gene

An international research team, led by Erwin Schurr and Thomas Hudson from the Research Institute of the McGill University Health Centre in Canada (http://www.muhc.mcgill.ca), has identified a gene on human chromosome 6 that confers vulnerability to leprosy [5].

Leprosy is caused by infection with the bacterium *Mycobacterium leprae*, transmitted by direct personal contact or by contaminated respiratory droplets, although the clinical forms of leprosy only develop in people that are intrinsically susceptible to the disease. Effective chemotherapeutic treatment is available for infected individuals; if the disease is left untreated, however, it could result in gross disfiguration.

Using linkage analysis of microsatellite markers to identify areas of the genome associated with susceptibility to leprosy, the research team analyzed DNA samples from 205 siblings from 86 families in Southern Vietnam that were susceptible to the disease. After finding a common gene variant on chromosome 6 they analyzed the DNA of an additional 208 families with leprosy to confirm their findings.

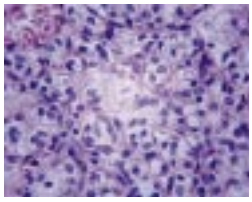
'This discovery will now allow us to study how the gene works and how it

influences the infectious process. This is an important step towards the development of innovative prevention and treatment strategies for leprosy', stated Schurr.

This is the second study from researchers at the McGill University Health Centre that illustrates the importance of host genes in infectious diseases 'We are now looking forward to applying the same gene identification strategies to other common infectious diseases, such as tuberculosis and malaria', stated Alexandre Alcaïs, a scientist at the Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 550 at Necker Medical School, Paris, and co-author of the study.

5 Mira, M.T. *et al.* (2003) Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat. Genet.* 33,412–415

Tumour-inducing cells get singled out



Of all the neoplastic cells in human breast cancers, only a small minority – perhaps as few as one in 100 – appear to be capable of forming new malignant tumours, according to recent research by scientists at the University of Michigan Comprehensive Cancer Center (http://www.cancer.med.umich.edu/) [6]. This discovery could help researchers home in on the most dangerous cancer cells to develop new, more effective treatments.

All cancer cells have a unique pattern of proteins, similar to a fingerprint, on their surface membranes. Using specific antibodies and flow cytometry technology researchers were able to segregate the phenotypically heterogenous cancer cells within a tumour into isolated populations based on their surface proteins. These isolated cell populations were then individually injected into immune-deficient mice and the mice were examined for tumour growth. It was found that a small group of cells, with a phenotype common to all but one of the human tumours in the study, could form new cancers in mice. These cells all expressed a protein marker called CD44, in addition to having either very low levels or no levels of a marker called CD24. As few as 100–200 of these tumour-inducing cells, isolated from eight of nine tumours in the study, easily formed tumours in mice, whereas tens of thousands of the other cancer cells from the original tumour failed to do so.

'These tumour-inducing cells have many of the properties of stem cells,' said Michael Clarke, who directed the study. 'They make copies of themselves – a process called self-renewal – and produce all the other kinds of cells in the original tumour.' For the first time, this work has made it possible to define what are believed to be the important cells – the cells that determine whether the cancer will come back or be cured – and it should be possible to develop drugs specifically aimed at these tumour stem cells. Furthermore, it is likely that similar cells drive the development of other types of cancer and this work is likely to have much wider relevance.

6 Muhammed, A-H. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0530291100 (http://www.pnas.org)

Miscellaneous

A giant magnet to catch a protein

A unique, ultra high-field 12 Tesla-strength magnet – the most powerful magnet ever made for research into cellular proteins and DNA – arrived from the UK for installation at the Mayo Clinic (http://www.mayo.edu/) recently. The 12 Tesla (unit of magnetic flux density, describing magnet strength) magnet is 240,000 times more powerful than the magnetic field of the Earth and is the strongest magnet currently used in medical research.

Understanding how proteins work through the use of proteomics will help researchers understand the biological basis of disease and as a result lead to the development of new diagnostic tests and treatments that work at the protein level. A major challenge is that proteins are small 3D molecules that are always changing in function and expression, with many occurring in low concentrations. 'You'll have to catch them when you can', says David Muddiman, Director of the W.M. Keck Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Laboratory at the Mayo Clinic.

The 12-Tesla FT-ICR (Fourier Transform Ion Cyclotron Resonance) mass spectrometer will be used for identifying proteins, thus enabling researchers to understand their structures, functions, roles and changing nature, forming this newest frontier of medical science – proteomics. It is also expected to be able to determine the identity of a human proteome – ~25,000 proteins expressed in a human cell at any given time – in a single day!

'This unique and novel instrumentation will further strengthen Mayo Clinic's research activities in proteomics. It also will clearly allow us to advance molecular medicine, both at the basic-science and the patient-care levels', says Muddiman.

E. coli can be good guys too

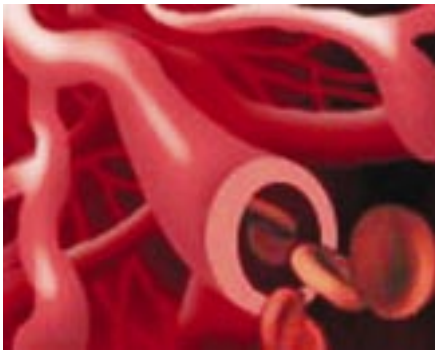
Escherichia coli toxins that cause diarrhoea can also inhibit the growth of metastatic colorectal cancer cells. GianMario Pitari and Scott Waldmann, both of the Thomas Jefferson University in Philadelphia (http://www.tju.edu/jmc/home/index.cfm) , and their colleagues at the Mayo Clinic in Rochester (http://www.mayo.edu/) have now shown just how these toxins elicit their anti-cancer effect [7].

Colorectal cancer, the fourth biggest cause of cancer-related death in the world, is most common in developed countries. It is known that the countries with the lowest risk of colorectal cancer are also those with the greatest incidence of enterotoxigenic *E. coli* infection. Enterotoxigenic *E. coli* produce heat-stable enterotoxins (STs), which interact with guanylyl cyclase C (GC-C), a receptor expressed by intestinal epithelial cells. This interaction leads to an increase in intracellular levels of cGMP, which activates cGMP-dependent guanylate cyclase (PKG) to cause diarrhoea. Waldman and Pitari have previously shown that interaction of STs with GC-C on colorectal cancer cells can also suppress the rate of the cancer cell division. Surprisingly, they have now shown this effect to be PKG-independent. Tests using an inhibitor of cGMP-gated channels and removal of Ca^{2+} both indicated that the slowing of cell division involves cGMP-gated Ca^{2+} influx. The discovery of this mechanism ties in with previous studies implicating dietary Ca^{2+} in the prevention of colorectal cancer.

'We propose blocking the pathway leading to diarrhoea and leaving only the positive effect', says Pitari. Because the toxin will not cross the intestinal lumen, intravenous infusion of the toxin should avoid the side effects of diarrhoea. 'This might provide a great opportunity to treat the cancer locally. It might also work synergistically with other anti-cancer drugs.' The team will start by looking at whether STs can inhibit growth of tumours in mouse models of human colorectal cancer.

7 Pitari, G.M. *et al.* (2003) Bacterial enterotoxins are associated with resistance to colon cancer. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2695–2699

Teaching an old drug a new trick



Researchers have shown in animal studies that a drug that has long been available in Europe can simultaneously block three of the four major biochemical pathways responsible for the blood-vessel damage that causes serious diabetic complications [8].

In people with diabetes, cells are bathed in blood that contains elevated levels of glucose. Most cells still manage to keep their internal glucose at normal levels, but certain cells – particularly endothelial cells that line arteries and the capillaries of the retina and kidney – are unable to regulate glucose. Instead, these cells develop high internal levels of the sugar, which they can not metabolize completely. As a result, glucose-derived metabolic intermediates accumulate and activate pathways of cellular damage that can eventually lead to diabetic complications.

Researchers from the Einstein Diabetes Research Center (http://www.aecom.yu.edu/home/) focused on two glucose-derived intermediates (fructose-6-phosphate and glyceraldehyde-3-phosphate) that activate three of these damaging biochemical pathways. Both of these metabolic compounds are the end products of another biochemical pathway mediated by the enzyme transketolase. The researchers reasoned that by boosting the activity of transketolase they might be able to reverse this pathway – essentially converting the two damage-triggering glucose metabolites into harmless chemicals and preventing all three damaging biochemical pathways from being activated.

Transketolase is dependent on the cofactor thiamine for its activity, but adding standard thiamine boosted transketolase activity by only 20%. The researchers then tried the drug benfotiamine, a fat-soluble thiamine derivative. This drug has been available in Germany for more than a decade where it is used to treat diabetic neuropathy, sciatica and other painful nerve conditions. 'By pure serendipity, it turned out that benfotiamine boosted the activity of the enzyme transketolase by 300–400% – something we never could have predicted based on benfotiamine's chemical structure,' said Brownlee, senior author of the study.

Benfotiamine successfully blocked all three major destructive biochemical pathways in experiments with arterial endothelial cells. Further studies showed that this drug was able to normalize all three pathways in diabetic rats and prevented diabetic retinopathy in the animals. No drug for preventing the complications of diabetes is currently available and this work provides a promising lead.

8 Hammes, H-P. *et al.* (2003) Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat. Med.* 9, 294–299

Scorpions: two stings in the tail

Recent research has shown that scorpions do not produce just one type of venom, but instead produce a prevenom that, although still poisonous, is molecularly and functionally different from true venom [9].

Parabuthus transvaalicus is one of several scorpions that produce true venom that is medically important and which comprises water, proteins, salts, small molecules and peptides. When the researchers, led by Bora Inceoglu at the University of California, Davis (http://www.ucdavis.edu/), studied *P. transvaalicus*, they found that it produces a drop of transparent fluid from the tip of its tail just before the true venom, which is more opaque, is released. When this prevenom was administered to insects and mice, the researchers found that it was more effective than true venom at causing pain and paralysis, respectively. However, true venom was more lethal to both groups overall.

Inceoglu and co-workers analyzed the prevenom and found that it contained toxic levels of potassium salts combined with relatively low levels of proteins and peptides, some of which also occur in true venom and cause it to be toxic. Further analysis confirmed that the prevenom has a different molecular make-up compared with true venom.

But why should a scorpion produce two types of venom? The authors propose that prevenom is used as an effective predator deterrent, as well as to immobilize small prey items, such as insects. This enables the scorpion to avoid using the more metabolically expensive true venom until it is really needed, for example when dealing with a larger predator. Such a system fits well with the resource-poor environment in which most scorpions live. The researchers also suggest that the prevenom might be part of the mating behaviour of the scorpions and also that people who survive being stung by a scorpion are only injected with prevenom, rather than with the more lethal true venom.

9 Inceoglu, B. *et al.* (2003) One scorpion, two venoms, prevenom of *Parabuthus transvaalicus* acts as an alternative type of venom with distinct mechanism of action. *Proc. Natl. Acad. Sci. U. S. A.* 100, 922–927

News in Brief was written by *Matt Brown, Morag Robertson, Linsey Stapley, Bryony Whisker, Catherine Wild and Heather Yeomans*