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News in brief

Targets and mechanisms

Protein functions in yeast elucidated using microarrays

Scientists have developed a method to determine the function of all the proteins that comprise an organism's proteome, it was reported recently[1]. The technique, developed at Yale University (New Haven, CT, USA) and North Carolina State University (NCSU; Raleigh, NC, USA), is the first to characterize interactions between all of the proteins in a single organism.

After cloning 5800 yeast genes, the group overexpressed and purified the corresponding proteins and arranged them at high spatial density on slides, to form a yeast proteome microarray. Studies of the interactions between arrayed proteins and other proteins, DNA and phospholipids identified many new calmodulin and phospholipid-interacting proteins. Moreover, further analysis of the calmodulin-binding proteins revealed a common putative binding motif.

Ralph A. Dean, Professor of Plant Pathology at NCSU, believes that the function of proteins within the yeast proteome is likely to be relevant to humans: 'Much of the machinery that's in yeast is inside us, in terms of the basic biology that makes the cell work.' He adds that his new high-throughput procedure could be used to prepare 40,000 human proteins for analysis, and could subsequently be used to screen for protein–drug interactions and to detect post-translational modifications.

1 Zhu, H. *et al.* (2001) Global analysis of protein analysis using proteome chips. *Science* 10.1126/science.1062191 (http://www.sciencemag.org)

Puncturing pathogens could lead to novel smart drugs

Perturbing the cell walls of pathogens using peptide nanotubes could provide a novel approach to antibacterial drug design. Researchers at the Skaggs Institute for Chemical Biology [part of The Scripps Research Institute (TSRI), La Jolla, CA, USA] have created cyclic peptide nanotubes, which can stack inside the membranes of bacteria and puncture the cell wall, causing cell death[2].

The nanopeptides were synthesized by alternating two forms of amino acid, resulting in 6- and 8-residue cyclic-D,L-α-peptides. In this study, these peptides were shown to act preferentially on Gram-positive or Gram-negative bacterial cell walls compared with mammalian cells, increase membrane permeability, collapse transmembrane ion potentials and cause rapid cell death.

The nanopeptides have shown potent antibacterial activity *in vitro* and *in vivo* against several pathogens including methicillin-resistant *Staphylococcus aureus*, one of the most prevalent multidrug-resistant bacterial strains that causes hospital-acquired infections. M. Reza Ghadiri, Professor of Chemistry at TSRI, hopes that because these peptides are synthetic, bacteria will be slower to develop resistance to them.

2 Fernandez-Lopez, S. *et al*. (2001) Antibacterial agents based on the cyclic-D,L-α-peptide architecture. *Nature* 412, 452–455

Promising breakthrough in leukaemia drug discovery

Researchers have identified a receptor-tyrosine-kinase inhibitor that modulates the signaling of mutant *FLT3* genes in leukaemia[3]. Researchers at Johns Hopkins Oncology Center (JHOC; Baltimore, MD, USA) hope that this could transform the most lethal, and common, form of leukaemia (acute myeloid leukaemia; AML) to the most treatable form.

The FLT3 receptor is normally involved in the growth and maturation of healthy blood cells. In AML, however, patients acquire an abnormal *FLT3* gene that constitutively activates the receptor and promotes inappropriate cell growth.

In this study, scientists performed dose–response cytotoxic assays with a tyrosine-kinase inhibitor, AG1295, on primary blasts from AML patients. The level of cytotoxicity of this agent was compared with that of cytosine arabinoside, and correlated with the presence or absence of mutant *FLT3*. AG1295 was found to be specifically cytotoxic to AML blasts with the *FLT3* mutation, which suggests that these blasts contribute to the leukaemic process.

Chemotherapy cures <10% of patients with AML who have a mutated *FLT3* gene and, therefore, the prospect of therapies that can block the activity of this receptor is exciting. Director of the research, Donald Small (Associate Professor of Oncology, JHOC) says, 'This is the payoff of more than a decade of laboratory research to pinpoint the genetic alterations associated with this type of leukaemia. Now, as hoped, we have evidence that the very abnormalities that cause the disease to progress could provide part of the cure.'

3 Levis, M. *et al*. (2001) A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. *Blood* 98, 885–887

Heart and insulin-producing cells grown from embryonic stem cells

Two recent papers describe, for the first time, the growth of heart cells[4] and insulin-producing cells[5] from embryonic stem cells. Both papers are the work of researchers from the Technion-Israel Institute of Technology (Haifa, Israel).

In the first paper[4], scientists cultivated embryonic stem (ES) cells in suspension, plated them to form aggregates called embryoid bodies (EBs), and characterized the phenotypic properties of the cells. It was observed that spontaneously contracting areas appeared in 8.1% of the EBs, and that these areas were positively stained for markers of cardiac tissue, such as anti-cardiac myosin heavy-chain, anti-α-actinin, anti-desmin, anti-cardiac troponin I and anti-ANP (atrial natriuretic peptide) antibodies. Furthermore, electron microscopy revealed myofibrillar organization that is typical of early-stage cardiomyocytes. The researchers also performed RT-PCR on the EBs, which demonstrated the expression of several cardiac-specific genes and transcription factors.

Importantly, electrogram analysis and measurement of intracellular Ca^{2+} ion flux showed that these embryonic-derived cardiomyocytes also had the functional properties of early-stage cardiomyocytes. This was further confirmed by the observed positive and negative chronotropic effects that were induced by isoproterenol and carbamylcholine,

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respectively. This is the first time that functional heart tissue has been differentiated from human embryonic cells, rather than from mouse heart cells.

The second paper[5], reports that human embryonic stem cells can produce insulin, which could lead to a cure for type 1 diabetes. Type 1 diabetes usually results from the autoimmune destruction of pancreatic islet β-cells, which causes insulin deficiency and complete dependence on exogenous insulin treatment. The current treatment is pancreas or islet transplantation, but a lack of donors has led to the development of alternative therapies.

Pluripotent undifferentiated human ES cells were used in this study as a model system for lineage-specific differentiation. Under both suspension and adherent culture conditions, the group observed spontaneous *in vitro* differentiation that included differentiation into insulin-producing β-cells, as demonstrated by immunohistochemical staining for insulin. Furthermore, secretion of insulin into the culture medium only occurred in the vicinity of differentiated cells, and was concurrent with the appearance of other β-cell markers.

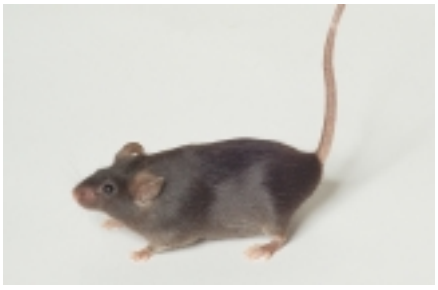
Christopher D. Saudek, President of the American Diabetes Association says, '[This study] offers the promise that stem cells might provide a rich source of insulin-producing cells and put us closer to a cure for this serious disease.'

4 Kehat, I. *et al*. (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J. Clin. Invest*. 108, 407–414

5 Assady, S. *et al*. (2001) Insulin production by human embryonic stem cells. *Diabetes* 50, 1691–1697

Murine model to test hepatitis C drugs

For the first time, a mouse model has been developed that can be used to test antiviral therapies against the hepatitis C virus (HCV)[6]. Up until now, only humans and chimpanzees have been susceptible to HCV and the development of effective therapeutics against the infection has been hampered owing to difficulties in establishing *in vitro* and *in vivo* small mammal models of viral replication. However, a group from the University of Alberta (Edmonton, Alberta, Canada) and Hôpital Saint-Luc (Montréal, Québec, Canada), led by David Mercer, has solved that problem.



The researchers generated chimeric mice by transplanting human liver cells into genetically modified mice (SCID mice) that carry a plasminogen activator transgene (*Alb-uPA*). The transplanted cells divide rapidly and fill up much of the mouse liver. These homozygous SCID/*Alb-uPA* chimeric mice can then be infected with HCV, support HCV replication within the human portion of their livers at clinically relevant titres, and transmit the infection to other chimeric mice.

The model will enable research into *in vivo* strategies for inhibiting viral replication or preventing infection by passive immunity and will advance the search for new antivirals and HCV vaccine development. The researchers have now founded a biotech spin-off company, KMTHepatech, which will actively use this model to work towards a cure for HCV.

6 Mercer, D. *et al*. (2001) Hepatitis C replication in mice with chimeric human livers. *Nat. Med*. 7, 927–933

Brain scanning: painful to watch

A new continuous brain-scanning technique has been used to observe, for the first time, the body's regulation of pain as it happens[7]. Levels of endogenous opioids (endorphins and enkephalins) were observed to increase greatly as patients experienced 20 minutes of sustained, slowly increasing, jaw pain.

The patients, participating in the double-blind, placebo-controlled study, had their brains simultaneously scanned by positron emission tomography (PET) to determine which areas were most neurochemically active. They then used a radiolabelled selective μ-opioid receptor ligand so that they could selectively measure the release of endogenous opioids and the activation of the μ-opioid receptors. The study showed an intense activation of the μ-opioid system in the amygdala, thalamus, hypothalamus, frontal cortex and nucleus accumbens.

'This result gives us a new appreciation of the power of our brain's own anti-pain system,' said Jon-Kar Zubieta, Assistant Professor of psychiatry and radiology at the University of Michigan Medical School (Ann Arbor, MI, USA) and assistant research scientist in the Mental Health Research Institute.

Opioids were already known to play a role in pain management but it has not previously been demonstrated live. The experiment also revealed that resting levels of opioids vary amongst individuals, suggesting a mechanism for how people experience pain in different ways. The improved knowledge of the mechanisms underlying chronic pain that can be gained from this experiment could help researchers ultimately find more effective treatments to relieve pain.

7 Zubieta, J-K. *et al.* (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* 293, 311–315

Apples reduce symptoms of chronic obstructive pulmonary disease

Catechin, a compound found in solid fruit, is beneficially associated with chronic obstructive pulmonary disease (COPD)[8]. The study of 13,651 Dutch adults from 1994 to 1997, of which 16% showed symptoms of COPD, showed that dietary levels of catechin correlated with a reduction in chronic cough, chronic phlegm and breathlessness, three symptoms of COPD. These and dietary intake were assessed by questionnaire.

Other flavonoid compounds, of which catechin is one, were also associated with improved health. Intake of flavonol and flavone positively correlated with increased pulmonary function (determined by spirometry), and decreased chronic cough and breathlessness.

8 Tabak, C. *et al.* (2001) Chronic obstructive pulmonary disease and intake of catechins, flavanoids and flavones. *Am. J. Resp. Crit. Care Med.* 164, 61–64

Could vitamin E and selenium prevent prostate cancer?

The largest ever prostate-cancer prevention study has just been launched by the National Cancer Institute. The aim of the study is to determine whether vitamin E and the trace element selenium can protect against prostate cancer – the most common form of malignancy in men, after skin cancer. The 12-year initiative will been overseen by the Fred Hutchinson Cancer Research Center (see http://www.fhcrc.org) and will be called the Selenium and Vitamin E Cancer Prevention Trial (SELECT). The trial will involve 32,400 men in the USA, Canada and Puerto Rico who will be recruited by a network of 400 research sites during the first five years of the trial so that they can be followed for at least seven years. The Hutchinson Center houses the SWOG Statistical Center, which designed the statistical structure of the study and will lead the data management and analysis.

Selenium (found in grains, meat and fish) and vitamin E (found in vegetable oil, dark green vegetables and whole-grain cereal) are both antioxidants that neutralize the effects of free radicals, known to damage the genetic material of cells, possibly leading to cancer. A previous study into the effects of selenium on skin cancer included results where, although it had no effect on the incidence of skin cancer, there was a reduction in the incidence of prostate cancer. Similarly, a study in Finland showed that although β-carotene seemed to increase the risk of lung cancer, vitamin E appeared to be associated with a reduced number of prostate cancers. The SELECT study will take the results of the previous studies further – it is the first study that will look at the effects of vitamin E and selenium (both separately and together) in the prevention of prostate cancer.

Clinical trials

Phase II trial shows NIL-A does not reverse Parkinson's disease

Amgen (Thousand Oaks, CA, USA) has completed its Phase II clinical trial of the neuroimmunophilin ligand (NIL-A) licensed to it by Guilford Pharmaceuticals (Baltimore, MD, USA), and has found that is does not reverse the motor symptoms of Parkinson's disease (PD).

The Phase II, randomized, double-blind, placebo-controlled safety and efficacy study of NIL-A is the first clinical trial of a neuroimmunophilin ligand for the treatment of PD. Three-hundred eligible patients (determined by the extent and severity of disease, their treatment and stability of symptoms) were randomly assigned to receive either placebo, 200 mg NIL-A or 1000 mg NIL-A four times daily.

During the study period, side effects, blood levels of NIL-A and changes in PD symptoms were evaluated. Single photon emission computed tomography (SPECT) was also used to measure the deterioration of nerve terminals in the brain region predominantly affected by PD. After 24 weeks, final clinical examinations were carried out, SPECT scans were obtained and treatment was discontinued.

Patients in the high-dose NIL-A group reported an increased incidence of transient nausea or indigestion. SPECT scans revealed that the mean percentage increase in dopamine nerve terminals was greater for patients in the high-dose group, and other measurements of PD symptoms were found to be significantly different between the placebo and high-dose groups.

However, analysis of these results, taking into account age, disease severity, duration of symptoms and type of anti-Parkinson's treatment, suggest that the NIL-A treatment regime studied in this trial is well-tolerated, but does not provide a substantial reversal of the motor symptoms of Parkinson's disease.

Secretin proves effective for autism

Patients treated with secretin show an improvement in the symptoms of autism after eight weeks, it was reported recently. Researchers from Repligen (Needham, MA, USA) presented the results of their Phase II trial of secretin at the *Annual Meeting of the Autism Society of America* (20 July 2001, San Diego, CA, USA).

Secretin was shown to significantly improve autism using the parental Clinical Global Impression Scale. Furthermore, the level of two biomarkers of autism (calpronectin, a marker of gastrointestinal inflammation, and chymotrypsin, a digestive enzyme) was found to be within the normal range in 51% of the patient population. Of these patients, further significant effects of secretin were observed on several endpoints including social function (as determined using the Autism Diagnostic Observation Schedule) and the Clinical Global Impression Scale (as determined by a professional and a parent).

New data was also presented on language function, as a fourth endpoint that is determined using the MacArthur Communicative Development Inventory. This endpoint assesses the number of words used and understood by children with minimal language capabilities. In a subgroup of 64 patients, secretin-treated patients had an average increase in receptive language of 33 words compared with a 6-word increase in the placebo group.

In addition to presenting its Phase II trial data, Repligen also reported the results of a meta-analysis of four, previously published, small Phase I studies. Responder data (defined using a Clinical Global Impression of Improvement, the Preschool Language Scale, parental reports of improvement and the Childhood Autism Rating Scale) were pooled from the four studies and demonstrated a significant increase in the rate of response to secretin compared with placebo.

Miscellaneous

UK pharma pays too much for foreign currency

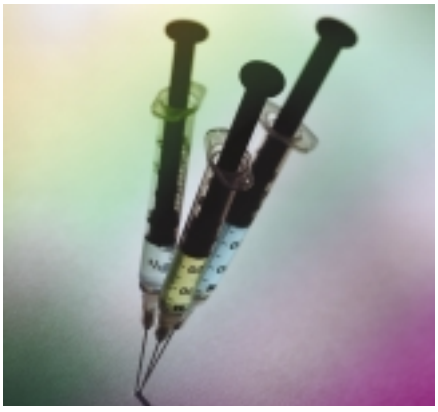
Failure to shop around for the best currency exchange rates is costing the UK pharmaceutical industry £280 million per year, announced a survey recently entitled '*The Real cost of money*', carried out on behalf of Moneycorp (London, UK). Thirty-seven percent of financial directors and controllers of companies surveyed (those with turnovers of up to £50 million per annum) made a transaction of £100,000 more than once a month, making the

potential loss through settling for poor exchange rates £280 million.

Forty-six percent of companies that responded cited convenience as the main advantage of using their bank. Eighty percent only bought or sold foreign currency through one bank, despite 30% saying that they believed specialist dealers provided better exchange rates. Furthermore, 32% only bought currency during normal banking hours, despite the availability of 24-h banking.

'With margins so keen in the pharmaceutical industry, bad currency practice… can mean the difference between success and failure,' said a Moneycorp spokesman. The survey also showed the increasing impact of the Euro. Fifty-one percent of companies said they bought it most often compared to seventy percent for the US dollar and nearly half the responders believe that the Euro will make their lives easier.

Syringe use set to fall as manufacturers push new insulin delivery products



Sales of insulin syringes will decrease over the next six years as the makers of more sophisticated reusable devices push their new products, revealed a new study by Frost & Sullivan (London, UK).

Manufacturers of reusable devices are expected to offer their products free to new diabetics in an attempt to gain as large a part of the US$438.9 million European diabetes drug delivery market as possible. Reusable pens are likely to become the largest selling drug delivery products in the UK, French, Italian and Spanish markets.

Sales in Germany are expected to differ from the common European model because of their emphasis on benefit over cost. Pump delivery products, which are viewed as superior to syringes and pens, will be most popular. A baseline level of syringe use will remain because a subsection of German patients remain loyal to older technologies.

Although growth in the number of diabetes sufferers from 175 million worldwide to an estimated 239 million in 2010 indicates sales growth, needleless devices will pose a great threat to reusable manufacturers. This technology could be 'drastic to device companies with an interest in the syringe and pen delivery segments,' said Jason Dabek, European industry manager for Frost & Sullivan's Global Diabetes Subscription Service.

European proteomics facility opens to research community

A new proteomics facility, based in Heidelberg, Germany, has recently been opened to all members of the European Molecular Biology Laboratory (EMBL) community. The facility aims to provide biochemists with experience of state-of-the-art proteomics technology and to demonstrate the substantial changes that proteomics is bringing to biological research.

'Two teams of researchers will be able to attend the visitor's centre for periods of approximately two weeks,' said Matthias Wilm, of the EMBL, who will supervise the facility. In this time they will be able to gain 'sufficient insight into how proteomics techniques can shed light on their challenging scientific questions,' he said.

Four scientists will be available to assist visiting researchers in using the technology.

GlaxoSmithKline sell Affymax

GlaxoSmithKline (GSK; London, UK) is selling the drug discovery company Affymax Research Institute (Palo Alto, CA, USA) to a syndicate led by Apax Partners Funds and Patricof & Co. Ventures (London, UK). The purchasing syndicate, which also includes the venture capitalists The Sprout Group and MPM Asset Management, is expected to invest US$51 million in the company. The terms of the sale are not yet definite but GSK is expected to receive non-voting preferred stock for their interest in the company.

'Affymax has transformed itself from a technology-development centre, focused on combinatorial chemistry and high-throughput molecular screening, to a drug-discovery company,' said Tadakata Yamada, Chairman of R&D at GSK. 'We have therefore decided to set that enterprise on a course towards independence, and invest the resultant savings for GlaxoSmithKline in the next innovative steps we intend to take in advancing healthcare,' he said.

Affymax should have a distinct advantage over its competitors because its technologies have been developed in conjunction with chemists and biologists at GSK. 'No other drug company has had this level of insight into the discovery process of a leading pharmaceutical company,' commented Lori Rafield, General Partner of Patricof & Co, who will sit on the Affymax board.

New joint program between FDA and NCI

The Food and Drug Administration (FDA; Rockville, MD, USA) and the National Cancer Institute (NCI; Bethesda, MD, USA) are to collaborate on a new research program, which will for the first time apply proteomics directly to patient care. The Clinical Proteomics Program will be funded for three years at US$1.1 million per year and aims to apply proteomics technologies, developed through previous collaborations between the two agencies, directly to patients.

Potential benefits of this program could include the development of individualized therapies that have been predetermined to be effective for each patient; determination of efficacy and toxicity prior to use in the clinic; and better understanding of the role proteins have in tumours. 'The great challenge now in proteomics is to begin to apply these technologies to clinical care,' said Emanuel Petricoin of the FDA's Center for Biologics Evaluation and Research (CBER).

One of the new techniques being used enables pure normal cells, pre-cancerous cells and tumour cells from the same patient to be isolated while maintaining the original protein pattern of the cell. This has previously not been possible and will enable researchers to study the effect of treatments on the pattern of proteins in a cell.

News in Brief was written by *Ben Ramster*, *Suzanne Berry* and *Joanna Owens*