News in brief

Targets and mechanisms

Combating Alzheimer's disease with vitamins

New research has provided evidence for a link between deficiencies in vitamin B_{12} or folate and an increased risk of developing dementia and Alzheimer's disease. Published recently in *Neurology*[1], the findings of Wang and colleagues are derived from the Kungsholmen Project, a population-based longitudinal study in Sweden.

In their report, the researchers followed a random sample of 370 subjects without any evidence of dementia, aged 75 years and older, and not taking vitamin B_{12} or folate supplements, for three years to detect cases of Alzheimer's disease. Of the 60 people who were diagnosed with having Alzheimer's disease, 26 (43%) had low levels of either vitamin B_{12} or folate; moreover, the risk of developing the disease was twice as high in subjects with deficiencies of these vitamins.

The mechanisms that underlie this potential interaction remain unclear, but decreased levels of vitamin B_{12} or folate can increase homocysteine levels which, in sufficient quantities, is a neurotoxic agent that can lead to cell death and neurological disorders such as Alzheimer's disease.

If such an involvement of vitamin B_{12} and folate in Alzheimer's disease is established, more careful monitoring of the levels of these vitamins in the elderly could represent one method of reducing the incidence of this disease.

1 Wang, H-X. *et al*. (2001) Vitamin B_{12} and folate in relation to the development of Alzheimer's disease. *Neurology* 56, 1188–1194

Synthesis of putative anticancer agent

The chemical synthesis of azinomycin A, a potentially revolutionary antitumour agent, has eluded scientists since its discovery in 1986, until now. The breakthrough by Coleman and coworkers[2] at Ohio State University (Columbus, OH, USA) has opened the door for the potential development of more effective anticancer drugs. By fragmenting azinomycin A into five simpler structures, the researchers were able to synthetically re-build each piece of the molecule, atom by atom.

Originally isolated from a fermented broth of the fungus *Streptomyces griseofuscus*, azinomycins A and B have been shown to be effective at combating tumour cells both *in vitro* and, more importantly, in mouse models of cancer.

The capability of the azinomycins to function as antitumour agents appears to be achieved by their ability to attack and bond with DNA in the major groove, a characteristic displayed by only a few substances. Detailed investigation into the full biological activity of the azinomycins and effectiveness in humans has been hindered by their instability and poor availability. It is anticipated that the synthesis of azinomycin A will assist in overcoming these limitations and help to resolve its antitumour mechanism of action, which will encourage the design of novel chemotherapeutic tools.

2 Coleman, R.S. *et al*. (2001) Total synthesis of azinomycin A. *Angew. Chem.* 40, 1736–1739

Smart worms lead to treatments for cognitive diseases

A mechanism of associated learning has been found to be calcium-dependent, involving a neuron-specific calcium sensor. Researchers at Roche (Basel, Switzerland) have used the nematode *Caenorhabditis elegans* as a model organism to study learning and memory with a view to applying this knowledge to the development of treatments for cognitive diseases, such as Alzheimer's disease, depression and schizophrenia.

Patrick Nef (Vice-President and Head of Molecular Neurobiology at Roche) says that: '*C. elegans* and human neurons share molecular synaptic components such as receptors, transmitters and ion channels, and enzymes with similar functions.'

By studying the learning of a skill, in this case the presence of food at a given temperature, researchers found that this learning process was calcium-dependent and required the functional neuron-specific calcium sensor-1 (NCS-1) in a pair of interneurons called AIY. Furthermore, the studies showed that overexpression of NCS-1 resulted in faster learning, an enhanced performance level and a longer memory. NCS-1 is highly conserved between *C. elegans* and humans and, therefore, is a potential therapeutic target and demonstrates the use of *C. elegans* as a model organism with which to validate drug targets.

Formation of RNA structure unfolded?

Researchers have successfully unfolded and refolded RNA molecules by applying mechanical force. Investigators at the Howard Hughes Medical Institute at the University of California at Berkeley (CA, USA), used precise mechanical forces to induce the folding and refolding of a variety of RNA molecules: a simple hairpin RNA, a molecule containing a three-helix junction, and the P5abc domain of the *Tetrahymena thermophila* ribozyme[3].

The three-dimensional structure of macromolecules such as RNA is crucial to their function and, therefore, expanding knowledge in this area is vital to enable the exploitation of these molecules as therapeutic targets. Furthermore, the mechanisms by which cells unfold proteins and RNA during degradation are as yet unknown.

Scientists have traditionally studied protein and RNA folding by chemical or temperature-mediated denaturation. However, the accuracy of this approach is limited by the large number of molecules studied and the fact that each molecule might unfold via a different mechanism.

This latest technique involved measuring the force required to unfold a single RNA molecule by attaching the end of each test molecule to a microscopic plastic bead. The researchers then used an 'optical trap' consisting of a laser beam that held and measured the force on one bead, and a piezoelectric actuator attached to the other molecule applied the precise forces required to unfold the molecule. This technique was also used to measure the length of the molecule as it was unfolded. The results of these experiments revealed that each molecule type studied had individual folding characteristics.

Carlos Bustamante, investigator in this study, would like to be able to use this research to provide biochemists with an energy function curve for RNA molecules,

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which will describe the energy barriers holding the molecules together and what is needed to unfold it. It is hoped that the mechanical unfolding technique could improve theoretical models of molecular folding, by enabling comparisons between predicted folding with that determined experimentally.

3 Liphardt, J. *et al.* (2001) Reversible unfolding of single RNA molecules by mechanical force. *Science* 292, 733–737

Prevention of HIV dementia by antioxidants?

The findings of a recent study on living brain cells could offer new hope towards reducing the incidence of dementia in patients with HIV. Presenting at the *53rd Annual Meeting of the American Academy of Neurology* in Philadelphia, PA, USA (5–11 May 2001), Avindra Nath of the University of Kentucky (Lexington, KY, USA) showed that several antioxidant drugs could protect brain cells from the damaging effects of HIV.

In the study, neurons from healthy subjects were cultured with cerebrospinal fluid from HIV patients with and without dementia, or control donors, and the functioning of the cells' mitochondria was monitored. The team found that the level of mitochondrial activity was compromised by HIV infection, whereas the extent of damage to the cells was concomitant with the severity of dementia of the patient. However, the most notable finding was that addition of various antioxidants to the cultures protected the cells from the toxic effects of the cerebrospinal fluid; the antiretroviral compounds, L-deprenyl and didox, represent two of the compounds of interest.

It is believed that dysfunction of the mitochondria can lead to numerous problems including cognitive impairments, which could underpin dementia. Although thorough clinical investigation of the potential therapeutic uses of these antioxidant agents is necessary, the results of this study could lead the way towards more effective prevention of dementia in HIV suffers.

Gene therapy restores sight in blinded dogs

Dogs suffering from an inherited form of retinal degeneration have had their vision restored by gene therapy. This treatment is the first example of effective gene therapy to treat blindness in large animals, and provides hope for humans with similar degenerative conditions. Researchers at the Institute for Animal Health, Cornell

Markets

Pharma could come to depend on US for profits

European initiatives to reduce drug prices are increasing the industry's dependence on the US for profits, concludes a recent study from Cambridge Pharma Consultancy (Cambridge, UK). Forty percent of global pharmaceutical sales come from the US as many medicines are launched in Europe at discounts of 60% of the US price. Organizations such as the National Institute for Clinical Excellence (NICE, London, UK) has contributed to reducing prices in the UK. Other European countries, such as Belgium, are also following the example of the UK, Spain and France in demanding capital back from the industry if they deem sales to be excessive.

These measures are making Europe a harder place than the US to operate. 'I am concerned about [European] governments applying short-term [cost-cutting] policies,' said Jean-Pierre Garnier of GlaxoSmithKline (London, UK). Prices in Europe, which account for a quarter of global sales (US$337 billion), are currently lower than in Japan, despite a decade of price cuts there.

Record year for European biotech but US still far in front

The European biotech industry has had its best ever year in 2000, but still fell farther behind the US which was flooded with new capital, concluded Ernst and Young's recent eighth annual European life sciences report entitled *Integration*. Fundraisings, valuations, revenues, employment and the number of companies present in Europe all reached record levels. A global trend of greater interaction between biotech companies was reflected in alliances, mergers and acquisitions, which were up 54% on 1999. This was no more relevant than in Europe where the report identified a need for companies to increase in size.

'[European biotech] is an industry that is characterized by huge resource demands,' said Glenn Crocker of Ernst & Young. 'In general, the more resources a company can command by virtue of its size, the quicker it can stake out a leadership position. Second place is rarely good enough in this industry.'

The UK dominates the European sector with over three times as many public biotech companies as any other European country (48 out of 105). Almost a quarter of Europe's biotech revenue (2 billion Euros) are from UK companies. However, the gulf between this and the US market is illustrated by the US life sciences company Amgen (Boulder, CO, USA) whose market capitalization is 70 billion Euros, almost equal to that for the whole of Europe. One suggested way of increasing Europe's stock might be through creating a single pan-European high-tech exchange, similar to the NASDAQ in the US.

To help the biotech industry in the UK, the EA Technology (Capenhurst, Chester, UK) has recently announced it will be investing £10 million in maximizing the commercial benefits arising from biotech in the UK. The Pro-Bio Faraday Partnership, who are coordinating the initiative, has received an initial £2.2 million grant from the Department of Trade and Industry and the Engineering and Physical Sciences Research Council (EPSRC; UK). The remaining money will be raised over the next five years from other research funds, development agencies and industry.

The initiative has the backing of nine universities and institutions, led by University of Manchester Institute of Science and Technology (Manchester, UK), the University of Cambridge (Cambridge, UK), University College London (London, UK) and the University of Edinburgh (Edinburgh, UK). Meanwhile, industrial support has been pledged by GlaxoSmithKline, AstraZeneca and DuPont Pharmaceuticals.

'Our aim is to bring together the people making rapid advances in bioscientific research with the industries who stand to gain most from exploiting the knowledge: in particular, high-value specialty chemicals, pharmaceuticals, agrochemicals and food', said David Gardner, Director of Pro-Bio.

University (Ithaca, NY, USA), the Schele Eye Institute and Department of Ophthalmology, University of Pennsylvania (Philadelphia, PA, USA) and the Department of Opthalmology and Powell Gene Therapy Center, University of Florida (Gainesville, FL, USA), have published these findings in *Nature Genetics*[4].

The retinal pigment epithelium (RPE) controls the normal function of the retina, and the pathogenesis of retinal degeneration. Mutations in the *RPE65* gene in Briard-beagle puppies results in the production of a non-functional form of RPE65 protein, which causes early loss of vision similar to that seen in Leber congenital amaurosis, a disease that causes near-total blindness in infant humans. They conclude, therefore, that *RPE65*-null Briard beagles represent a good model with which to study this severe degenerative disorder.

In this study, *RPE65* genes from healthy dogs were delivered using viral vectors into the subretinal space of the eye in Briard beagles known to have the defective *RPE65* gene from birth. Within only six weeks, the treated eyes were found to express the functional form of RPE65 and, after six months, tests including electroretinography, pupillometry and obstacle avoidance in dim light demonstrated that vision had been restored. These results will expand possible treatments for a variety of retinal diseases and the researchers are now evaluating patients who could potentially benefit from this discovery.

4 Acland, G.M. *et al.* (2001) Gene therapy restores vision in a canine model of childhood blindness. *Nat. Genet.* 28, 92–95

Antioxidant protects against radiation side-effects

An antioxidant compound, produced by Incara Pharmaceuticals Corporation (Research Triangle Park, NC, USA), has been shown to be effective at protecting normal tissues from the unwanted effects of radiation therapy. Zeljko Vujaskovic of Duke University (Durham, NC, USA), presenting at the *48th Annual Meeting of the Radiation Research Society*, reported that one of Incara's antioxidant drugs prevented damage to healthy lung tissue following radiation treatment in animal models of cancer. The small-molecule catalytic antioxidant was also found to exacerbate the antitumour actions of radiation and to inhibit tumour growth in non-irradiated skin and breast cancers.

In a separate preclinical study, communicated by Stephen Sonis (Brigham and Women's Hospital, Boston, MA, USA) at the *European Conference for Oral Oncology*, the same antioxidant was found to protect against mucositis, a side-effect of radiation found in ~40% of patients and characterized by painful mouth ulcers. By mopping up and inactivating many classes of free radicals, which are produced by radiation treatment, the catalytic antioxidant is thought to protect cells from their damaging effects.

The drug is awaiting clinical evaluation, but a compound that both successfully reduces tumour growth and protects healthy tissues would be a welcome development in the search for better cancer therapies.

Latest developments in stem cell research

A bone marrow stem cell that can transform into almost any organ has been discovered by researchers at the New York University School of Medicine (New York, NY, USA), Yale University School of Medicine (New Haven, CT, USA) and Johns Hopkins School of Medicine (Baltimore, MD, USA)[5].

Previously, it was thought that bone marrow cells were committed to making blood cells only, but over the past three years, the cells have been shown to be able to differentiate into mature epithelial cells of the liver, lung, gastrointestinal tract and skin. First, stem cells in the bone marrow of male mice were purified, and then a single cell was transplanted into female mice of which the bone marrow had been destroyed by radiation. Eleven months later, using Y-chromosome-specific dye, the male chromosome was found in tissue from the lung, oesophagus, stomach, small and large intestine, liver and skin, as well as in the blood and bone marrow.

The stem cells could be recruited to a damaged organ where, in response to certain signals, they will develop into the tissue of that organ. It is thought that any healthy cell with an intact genome can be reprogrammed to become a stem cell. An area of potential application is in therapy after toxic cancer treatments to repair damaged tissue, as well as in the treatment of diseases that involve tissue and organ damage, such as diabetes and cystic fibrosis.

In another study, the therapeutic potential of stem cell transplantation has been demonstrated in the treatment of spinal cord injury. The results of the study were presented at the *69th Annual Meeting of the American Association of Neurological Surgeons* (23 April 2001, Toronto, Canada) and show the effect of adult mouse brain-derived cell transplantation into the subacutely-injured adult mouse spinal cord. In a controlled study, eight out of 15 adult female mice with spinal injury received cell transplants and, seven days later, the mice that had received the transplants were found to show significant functional neurological recovery compared with the control animals. It is hoped that further studies will eventually lead to clinical trials in humans with traumatic and degenerative CNS injuries.

Another recent report from the Rockefeller University (New York, NY, USA) and the Sloan-Kettering Institute (New York, NY, USA)[6] describes how nuclear transfer can be used to differentiate embryonic stem (ES) cells generated from adult somatic cells. The group has developed microinjection technology a step further – to produce ES cells from the inner cell mass of a mouse embryo. Nuclear transfer was used to derive 35 ES cell lines from adult mouse somatic cells of various strains of mice (ntES cell lines). When the ntES cell lines were implanted into another embryo, they differentiated to all somatic cell types of the resulting mouse. This is the first study to show the potential of therapeutic cloning.

5 Krause, D.S. *et al.* (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105, 369–377

6 Wakayama, T. *et al.* (2001) Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. *Science* 292, 740–743

Killing viruses naturally

Researchers have discovered that NK cells can act specifically against certain viruses[7]. The group found that a genetic locus, the NK gene complex, controls resistance or susceptibility to murine cytomegalovirus (MCMV).

NK cells are known to act non-specifically by secreting interferon γ, which triggers further immune responses, but this group discovered that NK cells also respond specifically against certain pathogens. Humans who do not have NK cells are susceptible to only some viruses. Strains of mice that are normally resistant become susceptible on NK cell-depletion while other strains of mice are genetically susceptible. These researchers found an unusual mouse that had the genes for resistance but were still susceptible to virus infection. They found that these mice were lacking a receptor called Ly-49H. Normal mice that were given an antibody specific to Ly-49H to inactivate it were found to show the same degree of infection as mice in which all the NK cells had been inactivated.

The antigen receptors of B and T cells coupled with an immunoreceptor tyrosine-based activation motif (ITAM) were found to stimulate signalling cascades. Ly-49H also requires an ITAM-containing transmembrane molecule, which means that NK cells are more similar to T and B cells than was previously thought.

It is hoped that understanding how NK cells act against virus-infected cells will help in the development of specific vaccines.

7 Brown, M.G. *et al.* (2001) Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* 292, 934–937

Miscellaneous

New business patent places emphasis on product safety

Classen Immunotherapies (Baltimore, MA, USA) have registered a US patent (6,219,674), which is expected to create financial incentives for pharmaceutical companies that test their products and discover new adverse event information, and penalize those who do not.

Manufacturers of branded products are currently required by law to provide labels with their products, warning of adverse side-effects. However, beyond this, there is a reluctance to provide more detailed information because of the costs involved in research and the negative effect that information could have on sales.

Under the new business method patent, if a generic competitor discovers and patents a new adverse event associated with a brand name product, the generic competitor can force the manufacturer to remove the product from the market or give the generic competitor a license to manufacture it themselves. The latter situation could see a generic copy of a drug appearing 3–4 years after the introduction of a new drug. Consumers are expected to benefit from the more comprehensive safety warnings, as are generic manufacturers if they successfully license new adverse events.

Judge dismisses court case against makers of Ritalin

US District Judge Rudi M. Brewster reaffirmed his earlier ruling of 8 March 2001 to dismiss the class action lawsuit filed against Novartis Pharmaceuticals (East Hanover, NJ, USA). The lawsuit claimed that the company conspired with the American Psychiatric Association (APA) and Children and Adults with Attention-Deficit/Hyperactivity Disorder (CHADD) to promote the diagnosis of attention deficit disorder/hyperactivity disorder (ADHD).

The case was dismissed under California's anti-SLAPP statute that provides for the dismissal of lawsuits that attack defendants for their speech about an issue of public interest. On the earlier occasion, the Judge held the plaintiff's complaint so vague and unclear that it did not constitute a legal claim.

'We are heartened that an overwhelming body of scientific evidence cannot be litigated away by lawyers and antipsychiatry advocates,' said Dorothy Watson, Novartis General Counsel. The lawsuit, and other similar suits filed in Texas, Florida, New Jersey and Puerto Rico claim that Novartis conspired with the APA and CHADD to broaden and expand the diagnosis and diagnostic criteria for ADHD.

TIGR awarded grant to research *Cryptococcus neoformans*

The Institute for Genomic Research (TIGR, Rockville, MD, USA) has received funding from the National Institute of Allergy and Infectious Diseases (NIAID; Bethseda, MD, USA) to sequence the genome of the fungus *Cryptococcus neoformans*. The grant will enable researchers to provide 4–5-fold sequence coverage of the *C. neoformans* genome, to generate an additional fourfold sequence coverage, to assemble all the sequence data made currently available from the work of Ronald W. Davis and Richard Hyman (both of the Stanford Genome Technology Center, Stanford University, CA, USA) with that at TIGR, and complete the gap closure as fully as possible.

Researchers at TIGR will then focus on identifying the open reading frames (ORFs) in the genome using a variety of computational methods. Once complete, the data will be the first basidiomycetes genome to become publicly available. *C. neoformans* is a globally relevant fungus because it is the aetiological agent of cryptococcosis, one of the most common pathogens isolated in the CNS today.

Bank accused of caving in to extremists

The Association of Medical Research Charities (AMRC, London, UK) has removed its custom from the HSBC Bank in protest at the bank severing its own ties with the animal research establishment Huntingdon Life Sciences (HLS, Cambridge, UK). The establishment has recently run into a funding crisis following sustained action from animal rights organizations, which accused it of cruelty to animals.

The AMRC, whose 112 members hold approximately £16 billion of assets, has however asserted its belief that animal research is essential to science and has accused the bank of 'caving in to extremists'. Meanwhile, the Wellcome Trust is also considering its future ties with the bank. Several other financial institutions in the UK have also ceased dealing with HLS since being threatened by animal rights groups.

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