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News in brief

Targets and mechanisms

Key protein identified in atherosclerosis

Removal of the cell adhesion protein CD44 has been shown to reduce the incidence of atherosclerosis in mice by 50–70%[1]. Using mice without the *CD44* gene (making them prone to developing the disease), research showed that the protein, expressed on inflammatory and vascular cells, supports the adhesion of activated lymphocytes to endothelial and smooth muscle cells by promoting the recruitment of macrophages to atherosclerotic lesions.

'It suggests that if we can develop drugs to interfere with CD44 or, perhaps, selected molecules with which it interacts, we may be able to make a real contribution to lowering the likelihood of heart attack and stroke in at-risk individuals,' said Ellen Puré, of The Wistar Institute (Philadelphia, PA, USA) and senior author of the study.

1 Cuff, C.A. *et al.* (2001) The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *J. Clin. Invest.* 108, 1031–1040

Hottest genome sequenced

The genome of the world's highest-temperature organism, the bacterium *Pyrolobus fumarii*, has been sequenced by Diversa Corporation (San Diego, CA, USA) in collaboration with Celera Genomics (Rockville, MD, USA). The bacterium, which lives in mid-Atlantic hydrothermal vents at temperatures of 90–113°C, is hoped will be a source of thermally stable agricultural, chemical and chiral pharmaceutical products. *P. fumarii* is also able to live on inorganic chemicals, hydrogen and carbon dioxide and is, therefore, expected to contain many novel metabolic enzymes of commercial interest.

The genome was found to be 1.85 Mbp in length and to contain ~2000 genes. Initial sequence annotation has revealed an unusually high number of genes with no obvious similarity to previously described genes from eubacteria and archaea.

Key angiogenesis gene identified

Scientists in the USA have found that overexpression of a specific gene in mice, hypoxia-inducible factor-1α (*HIF-1α*), can help generate new blood vessels without causing the side effects associated with previous attempts to promote angiogenesis[2]. This discovery could help in the search for novel therapies for cardiac and peripheral vascular disease, diabetes and recalcitrant wounds.

The HIF-1α protein is one subunit of the total HIF-1 protein, which regulates gene activity during angiogenesis in response to oxygen deprivation. The *HIF-1α* gene is normally overexpressed during carcinogenesis, wound healing and myocardial infarction.

Jeffrey Arbeit and colleagues at the University of California, San Francisco (CA, USA) generated a transgenic mouse strain that over-expressed *HIF-1α* specifically in epidermal cells to ensure high levels of active HIF in their skin. Arbeit found that these mice showed a 66% increase in dermal capillaries, a 13-fold rise in total vascular endothelial growth factor (VEGF) levels, and a 6–9-fold induction of each VEGF isoform.

Previous attempts to promote healthy blood vessel growth have been based on the over-expression of *VEGF*, a gene that lies downstream of *HIF-1*. However, although increased VEGF does induce angiogenesis, the new vessels tend to be leaky and oedema and inflammation are seen. These side effects were not noted in the *HIF-1α* transgenic mice thereby making this a possible candidate for tissue ischemia therapy.

2 Elson, D.A. *et al.* (2001) Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1α. *Genes Dev.* 15, 2520–2532

Popeye's food to aid the blind

Scientists have discovered that spinach, long seen as a strength-giving foodstuff, might prove beneficial to blind people. The research was performed by a team at the Department of Energy's Oak Ridge National Laboratory (ORNL; Oak Ridge, TN, USA) and the University of Southern California (USC; CA, USA), and was led by Eli Greenbaum of ORNL. The team proposed replacing dysfunctional photoreceptors with a protein found in spinach that emits a small electrical voltage after capturing energy from photons.

Greenbaum's collaborator, Mark Humayun (a professor in the USCs Doheny Eye Institute) and his coworkers, showed that if retinal tissue is electrically stimulated using electrodes implanted in the eyes of blind patients, many see images similar to those stimulated by light. The individuals who could gain most benefit from this research are those who suffer from retinitis pigmentosa, the leading cause of blindness, where the photoreceptor cells degenerate. In the USA, retinal degeneration has left 20,000 people blind and 500,000 people with visual impairment. Age-related macular degeneration, which is a disease that rarely leads to blindness but causes vision impairment by affecting the centre of the retina, could also be treated.

This protein, from photosystem I, could be used to restore the activity of these photoreceptors and can produce voltages of up to 1 V upon capturing the energy from light. Recent research has shown that the protein can be incorporated into the membrane of an artificial liposome system, which mimics the living cell. Greenbaum says, 'What we need to find out is whether these voltages can trigger neural events and allow the brain to interpret the images.' For more information see http://www.ornl.gov/news.

Viral targets and mechanisms

Is ALS the result of viral infections?

Scientists have discovered that the AIDS virus can cause a form of ALS, or amyotrophic lateral sclerosis (also known as Lou Gehrig's disease) that improves, or can even be resolved, upon treatment[3]. This research provides evidence supporting the debate about whether ALS can be caused by viral infections. There is currently no ultimate treatment for ALS, other than that which slows the progression of this disease.

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Neurologist Burk Jubelt[4] of the Upstate Medical University in Syracuse (SUNY; NY, USA) says, 'This is exciting news, because if this form of ALS caused by HIV is treatable, then other forms of ALS may be treatable as well.'

The study, performed in part at the Adolphe de Rothschild Foundation (Paris, France), focussed on 1700 HIV-infected patients, of which six developed neurological symptoms over the 13-year study period; this is much higher than in the general uninfected population. Following treatment with anti-HIV drugs, two patients recovered completely from the neurological disorders, three improved and one stabilized.

Daniel MacGowan, neurologist at the Beth Israel Medical Center (New York, NY, USA) describes the case of a 32-year-old woman with ALS-like symptoms, who was found to be HIV-positive[5]. Upon treatment with nelfinavir, zidovudine and lamivudine, the patients neurological disorder diminished, along with the plasma levels of HIV RNA.

Antoine Moulignier, lead author of the research performed in France, says more research is necessary to determine how HIV can cause motor neurone disease. 'It could be through several mechanisms – through neuronal infection, by secretion of toxic viral substances, by inducing the immune system to secrete cytokines, or by inducing an autoimmune disease.'

3 Moulignier, A. *et al.* (2001) Reversible ALS-like disorder in HIV infection. *Neurology* 57, 995–1001

4 Jubelt, B. and Berger, J.R. (2001) Does viral disease underlie ALS? Lessons from the AIDS pandemic. *Neurology* 57, 945–946

5 MacGowan, D.J. *et al.* (2001) An ALS-like syndrome with new HIV infection and complete response to antiretroviral therapy. *Neurology* 57, 1094–1097

The great escape

Researchers have found that the AIDS virus can overcome the normal machinery of a cell, thus leaving the cell to infect other cells[6]. The team, based at the University of Utah, and Myriad Genetics (both Salt Lake City, AZ, USA), silenced the gene that is usually responsible for the production of the Tsg101 protein. In the absence of this protein, HIV particles could not escape or 'bud' from the cells, leaving them unable to infect neighbouring cells. The leader of the study, Wes Sundquist, Professor of Biochemistry at the University of Utah, says, 'We showed the virus can't bud without the protein. Instead, the virus gets stuck at the last stage of leaving the cells.'

Researchers at Myriad Genetics have identified other proteins that work with Tsg101 to aid in viral budding and subsequent cell infection. Many of these proteins are 'likely to be good targets' for potential new therapies against HIV, says Kenton Zavitz, a molecular biologist at Myriad.

It was already known that part of the AIDS virus, the Gag protein, is essential in HIV budding between cells. This recent study has shown that Tsg101 connects to Gag, and other parts of Tsg101 interact with other proteins within the cell. Therefore, blocking transcription of the *tsg101* gene, blocks the linkage of the AIDS virus to the cells' particle-making machinery. This is the first study to show that the Tsg101 protein is involved in helping HIV to escape cells and spread to others. Further information is available from http://www.utah.edu/unews/releases/01/oct/aidsbud.html.

6 Garrus, J.E. *et al.* (2001) Tsg101 and the vacuolar protein-sorting pathway are essential for HIV-1 binding. *Cell* 107, 55–65

How viruses take hold of cells

Researchers have elucidated the molecular-level interaction between coxsackievirus – which can cause infections of the heart, brain and pancreas – and its human cell protein receptor[7]. This was part of ongoing work at the US Department of Energy's Brookhaven National Laboratory (Upton, NY, USA) and Purdue University (West Lafayette, IN, USA) and could lead to improved methods of reducing viral infections, and help in the design of virus-based vehicles for gene therapy.

The coxsackieviruses (CVB) use the coxsackievirus-adenovirus receptor (CAR) to recognize host cells. The study revealed that the CAR forms pairs on the cell surface and, when the virus binds, it interacts with both receptors, decreasing the likelihood of virus release unless both bonds are broken simultaneously. Another feature of the interaction, which is advantageous to the virus, is the structure of the binding site, which is like a dimple on the virus particle surface that is inaccessible to the natural human antibodies but readily accessible to the receptor. These features are shared by other viruses in the same family, for example, the virus that causes polio and rhinovirus, and other respiratory and gastrointestinal infections.

Brookhaven biologist Paul Freimuth says, 'It's a very clever arrangement that these viruses have worked out, and very hard to defeat.' Although single receptor-like molecules have been used in an attempt to block the virus' attachment to cells, these have not met with much success. However, the application of receptor-like molecules with double binding-sites could be more efficient in interfering with the viral attack.

The data obtained were correlated with earlier work by Freimuth and coworkers[8], at Brookhaven's National Synchrotron Light Source, which showed a portion of the CAR bound to the adenovirus.

7 He, Y. *et al.* (2001) Interaction of coxsackievirus B3 with the full length coxsackievirus-adenovirus receptor. *Nat. Struct. Biol.* 8, 874–878

8 Bewley, M.C. *et al.* (1999) Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. *Science* 286, 1579–1583

Targets and mechanisms for neurodegenerative disorders

Biological marker found for PD

A previously unknown molecule has been found in key areas of the brains of patients with Parkinson's disease (PD). This finding could be particularly important for diagnosis of the disease because, currently, diagnosis is based strictly on clinical assessment. The molecule, known by its acronym ADTIQ, could herald new treatments for sufferers of the disease. The findings were presented at the *126th Annual Meeting of the American Neurological Association* (30 September–2 October 2001, Chicago, IL, USA).

PD is caused by a progressive loss of neurons in the substantia nigra. These neurons produce dopamine, which helps to direct muscle activity. As dopamine is lost, the clinical features of PD emerge, which include tremor at rest, muscle rigidity and slowness of movement.

Although scientists have not yet found the cause of neuronal death, a probable suspect is tetrahydroisoquinoline (TIQ), which has been found to be toxic to dopaminergic neurons in previous studies. The newly discovered molecule ADTIQ is structurally similar to the neurotoxin TIQ.

ADTIQ was found in elevated levels in all parts of PD brains examined, compared with brains of normal individuals. Significantly, high concentrations were found in the substantia nigra – the part of the brain where dopamine is used as a chemical messenger to communicate with other nerve cells.

Confirmation of these results would hail early diagnosis of PD, thereby enabling earlier intervention with therapies to slow or halt progression of the disease.

Highs and lows of AD

Suppression of hormones such as testosterone has been correlated to increased levels of blood amyloid – a molecule that has major implications in Alzheimer's disease (AD). The results of the study were presented at the *126th Annual Meeting of the American Neurological Association* (30 September–2 October 2001, Chicago, IL, USA).

AD is characterized by memory loss and other cognitive brain functions, such as reasoning. Accumulation of amyloid in the brain leads to senile plaques, which are toxic to neuronal cells. Doctors find high numbers of senile plaques in regions of the brain responsible for memory, indirectly implicating amyloid in the disease.

Previous studies in guinea pigs found that females who had their ovaries removed showed increased concentrations of amyloid in the brain. After receiving hormone replacement therapy, levels of brain amyloid dropped, suggesting that the hormones might help to break down amyloid in some way.

The reported study was in males suffering from prostate cancer who were receiving testosterone suppressors as part of their therapy. In each of the six men studied, plasma amyloid levels approximately doubled over the six-month duration of the study.

A large ten-year study investigating the effect of hormone replacement therapy on the prevention of AD is currently underway; five-year interim results are due in 2003.

New strategy for treating misfolding diseases

A new strategy that has the potential to treat familial amyloid polyneuropathy (FAP), a disease analogous with Alzheimer's disease (AD), has been uncovered[9]. The research, published in a recent issue of *Science,* could have important implications for treatment of the disease.

FAP is caused by misfolding of the protein transthyretin (TTR), which is produced by the liver. Normally, TTR is present in the blood as a tetramer made up of four identical protein subunits. Two different genes, located on two different chromosomes, encode these identical subunits. However, if one of the genes has a mutation, hybrid tetramers form, which are less stable and dissociate under certain conditions. The dissociated subunits can then misfold and reassemble into 'hair-like' amyloid fibrils. It is the accumulation of these fibrils around the muscles and peripheral nerve that causes FAP. The only current treatment for the disease is a liver transplant.

The study showed that by introducing a 'suppressor subunit' into a destabilized TTR tetramer, dissociation and subsequent misfolding into amyloid fibrils could be prevented. They found that incorporation of only one suppressor unit into the tetramer significantly increased its stability.

This approach could form the basis for a new therapeutic strategy for FAP patients, in which the patients receive an injection of the suppressor protein. Alternatively, future gene therapy techniques might enable the introduction of the suppressor gene directly into the organ enabling the protective subunit to be incorporated during TTR biosynthesis.

9 Hammarström, P. *et al.* (2001) *Trans*-suppression of misfolding in an amyloid disease. *Science* 293, 2459–2462

AD and PD proteins: a lethal combination

Interaction of the two proteins that cause Alzheimer's disease (AD) and Parkinson's disease (PD) could mean that current therapies that target each disease specifically might have a broader benefit across these patient groups[10]. AD and PD are associated with cerebral accumulation of β-amyloid and α-synuclein, respectively. However, some patients show features of both diseases, raising the possibility of overlapping pathogenic pathways.

To test this hypothesis, strains of mice were developed that produced either human amyloid precursor protein (hAPP) or human α-synuclein (hSYN), or both. Mice that produced both proteins had severe defects in learning and memory and, significantly, developed motor deficits earlier than mice producing α-synuclein-alone. It was also found that β-amyloid peptides promoted aggregation of a-synuclein in a cell-free system. The evidence suggests that β-amyloid might contribute to the development of diseases such as PD by promoting accumulation of α-synuclein.

If the interaction of these proteins is found to accelerate and exacerbate the symptoms of their respective diseases, then drugs preventing the accumulation of each protein could have broader implications than previously thought.

10 Masliah, E. *et al.* (2001) β-amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12245–12250

Miscellaneous

Traditional therapies still dominate despite attempts to diversify

The three main therapeutic areas of cardiovascular, CNS and infectious diseases will dominate sales of new blockbuster drugs by 2007, according to a recent Datamonitor (London, UK) report. The three areas will account for 58.9% of sales, whereas treatment of CNS disorders will account for the greatest number (6) of new therapies. This dominance comes despite the attempts of major companies to expand the breadth of their product portfolios.

Merck & Co. (Whitehouse Station, NJ, USA) have new products in the areas of anti-infectives, CNS, arthritis and pain (away from their previous focus on cardiovascular products). AstraZeneca (London, UK) have also moved away from their traditional focus on gastrointestinal products, entering instead the cardiovascular and oncology markets. These new products are expected to

compensate for the expected loss in 2007 of the US patent for Prilosec (omeprazole) by providing estimated new blockbuster sales of US$6340 million in 2007. Eli Lilly (Indianapolis, IN, USA) are also expecting to lose revenue when their patent for Prozac (fluoxetine) expires in mid-2001. They aim to counter this by launching three blockbuster products in 2007 with estimated combined sales of US$4050 million.

The drugs pipeline is not as promising elsewhere in the field. F-Hoffman-La Roche (Basel, Switzerland) and Bayer (Wuppertal, Germany) have spent US$2019 million and US$2095 million, respectively, in 2000 on R&D but have no new blockbuster products in late-stage development. Similarly, American Home Products (Madison, NJ, USA), Abbott Laboratories (Abbott Park, IL, USA) and Boehringer Ingelheim (Ingelheim, Germany) are predicted to suffer difficulties maintaining growth by 2007 unless they develop a large number of smaller products that can be marketed by then.

Vertex suspends development of kinase inhibitor

Vertex Pharmaceuticals (Cambridge, MA, USA) are to suspend clinical development of their lead orally active p38 MAP-kinase inhibitor VX745 because of a recent observation of adverse side effects in animal models. The side effects, exhibited in the CNS of one of two animal species used, were noted when using a dose ten-fold higher than that used in human clinical trials. Previously significant clinical effects had been shown by a low-dose 12-week randomized, placebo-controlled study for patients with rheumatoid arthritis.

Recruitment for a higher-dose patient trial has now been cancelled, as has a trial for myelodysplastic syndrome. They aim to publish Phase II clinical data in a peer-reviewed forum in 2002.

Vertex will instead concentrate on developing their other second-generation oral p38 MAP-kinase inhibitors, VX702 and VX850. Having done proof-of-principle studies with VX745, the company aims to initiate clinical trials in one or both of these other products in 2002.

Institute for Systems Biology gains US$828,500 grant for new equipment

The Institute for Systems Biology (Seattle, WA, USA) has received a US$828,500 grant from the M.J. Murdock Charitable Trust to purchase specialized robotics workstations and mass spectrometers for the preparation, processing and analysis of protein samples. The Institute, a private non-profit research organization, will use the money to develop new technology platforms based on the Isotope Coded Affinity Tag (ICAT) reagent method for measuring the levels of proteins in complex solutions. Ruedi Aebersold, developer of the method and leader of the Institute's proteomics research, said high-throughput quantitative proteomics was 'vital to realizing the hope and potential created by the Human Genome Project'.

Funding for cooperative Alzheimer's study doubled

The NIH National Institute of Aging (Bethesda, MD, USA) has doubled its funding of the Alzheimer's Disease Cooperative Study (ADCS), its research agreement with the University of California, San Diego (UCSD; CA, USA). The grant, of US$54 million over a period of five years, will fund the ADCS to continue coordinating clinical trials of new approaches to treating and preventing Alzheimer's disease.

'In the next ten years or less, we should be able to delay the onset of Alzheimer's disease,' said Leon Thal, Chair of the Department of Neurosciences at UCSD School of Medicine. He says that they have established a group of nationally and internationally recognized Alzheimer's researchers that is now large enough to generate significant results in a reasonable period of time.

The ADCS has researchers at 83 sites in the USA and Canada and has conducted 13 research protocols over the past ten years. Five other research protocols are planned for the next five years.

News in Brief was written by *Natalie Baderman, Joanne Clough, Joanna Milburn* and *Ben Ramster*

People

Awards

Three key awards from the Society for Biomolecular Screening

Leroy Hood (Institute for Systems Biology, Seattle, WA, USA) and Michael Hunkapiller (Apperla Corp and Applied Biosystems, Foster City, CA, USA) were awarded the SBS Achievement Award at the recent *Society for Biomolecular Screening* meeting in Baltimore (MD, USA) for their significant accomplishments in life science research. Meanwhile, Keith Wood of Promega Corp picked up the PerkinElmer Life Sciences Award for Innovation in Automation and High Throughput Screening for his research into bioluminescent reporter gene technology.

Hood and his past colleagues at the University of Washington played a key role in developing automated microchemical instrumentation for the sequence analysis of proteins and DNA and the synthesis of peptides and gene fragments. More recently, he has been involved in the analysis of the human and mouse T-cell receptor loci. Meanwhile, Hunkapiller and colleagues at Applied Biosystems developed and marketed the first automated sequencing machine in the mid-1980s. Hunkapiller's group has since developed the PR Prism 3700 machine, which was used for all of Celera's efforts to sequence the human genome and much of the public Human Genome Project.

Finally, Wood, together with a team from the University of California, cloned a gene responsible for the light in fireflies and demonstrated its potential for measuring events in living cells. Wood then

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