News in brief

Targets and mechanisms

Quorum sensing in bacteria

Scientists have identified a molecule that enables bacteria to send inter-species signals [1]. This could lead to the design of novel drugs that target and disrupt bacterial communication. The cell–cell communication of bacteria is accomplished through a process known as quorum sensing, which enables the coordinated expression of genes in bacterial populations.

The study, at Princeton University (Princeton, NJ, USA), centred on a signalling factor that was discovered to contain boron, and culminates years of research. In 1993, Bonnie Bassler (a biologist at Princeton and co-author of the recent paper) identified four genes that make ocean organisms emit a blue light; this triggered an interest in the system and how the bacteria respond to each other's signals [2].

Fred Hughson, who led the study, said: 'It is a detective story – one with a surprise ending.' The identification of the molecule that is key to quorum sensing has shown that at least 50 kinds of bacteria produce this molecule, autoinducer-2 (AI2), including ones that are responsible for human diseases, such as *Escherichia coli.* Hughson added: '[The bacteria] are biding their time until they grow up to some critical population density at which they turn on their virulence factors.'

The researchers present the X-ray crystal structure of AI2 complexed with a sensor protein, LuxP. The ligand is a furanosyl borate diester that has no resemblance to any previously characterized autoinducers. This also suggests a potential biological role for boron, whose presence was confirmed by NMR and whose requirement by several organisms had proved elusive.

Hughson explains that, now the composition of AI2 has been elucidated, pharmaceutical companies could attempt to develop analogues of AI2 that do not trigger the quorum-sensing response and would prevent bacteria from releasing harmful toxins. This would not be a typical antibiotic in that it would not kill the bacteria but would disrupt its activity. This could decrease the likelihood of these bacteria developing resistance, which is an increasing problem faced with current antibiotics.

1 Chen, X. *et al.* (2002) Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415, 545–549

2 Bassler, B.L. (1993) Intercellular signalling in *Vibrio harveyi:* sequence and function of genes regulating expression and luminescence. *Mol. Microbiol.* 9, 773–786

Structure of Eph–ephrin complex revealed

Scientists have revealed the molecular structure of the ligand–receptor interaction between the Eph receptor and its protein ligand, ephrin [3]. Using X-ray crystallography, the group at Memorial Sloan-Kettering Cancer Center (New York, NY, USA) obtained the first-ever detailed pictures of the proteins interacting with each other, thus providing information that could be useful for designing drugs for neurodegenerative and cardiovascular diseases, and cancer.

The Eph family of tyrosine receptor kinases and their membrane-anchored ligands are involved in regulating the cell–cell interactions that occur between a variety of cell types in many cellular processes. They do this by initiating a unique bidirectional signalling cascade to enable information to be communicated into both the Eph-expressing and ephrin-expressing cells. In particular, their role in the communication between vascular endothelial cells during angiogenesis could have great relevance to the progression of cancer. Hence, elucidating the structural detail of the Eph–ephrin complex might provide a new approach to the design of drugs that could block angiogenesis.

'Given the importance of Eph receptor kinases and ephrins in cardiovascular function, nerve regeneration and cancer, these results could be the first step towards the future development of novel therapeutic strategies,' said Dimitar Nikolov, Head of the Structural Biology and Neuroscience Laboratory at Memorial Sloan-Kettering Cancer Center.

3 Himanen, J-P. *et al.* (2001) Crystal structure of an Eph receptor–ephrin complex. *Nature* 414, 933–938

Enzyme links obesity and diabetes

Researchers have identified a mechanism that could explain how leptin acts to metabolize fatty acids in muscle tissue [4]. The study is the first to provide a molecular link between obesity and diabetes, and could therefore lead to new treatments for both conditions.

Since its discovery in 1994, leptin has gained much attention as an appetite suppressant, being produced in the muscle and then travelling via the bloodstream to receptors in the brain. However, the new research suggests the hormone also has a role to play in metabolism.

Fatty acid build-up in the muscle and liver impairs the ability of the body to burn calories and also leads to insulin resistance. The new study suggests that the enzyme 5-AMP-activated protein kinase (AMPK), which is known to be involved in cholesterol metabolism and glycogen synthesis, might also serve as a signalling pathway for leptin, enabling fatty acid metabolism.

In a mouse model, leptin activated AMPK in muscle when injected into a peripheral vein or the brain hypothalamus. The same effect was observed in muscle cells *in vitro*. In addition, blocking nerves to the animal's legs revealed that leptin exerted an indirect effect on muscle via the brain and sympathetic nervous system.

The results show that leptin resulted in an early activation of AMPK in skeletal muscle, but later inhibited another enzyme in the pathway, acetylCoA carboxylase (ACC), which resulted in increased fat metabolism. In addition, the study showed that only a single isoform ($\alpha 2$) of leptin is key to the effects of fatty acid metabolism. This highlights the AMPK pathway as a promising target for drug treatments for obesity and diabetes, and the molecular specificity identified in the $\alpha 2$ isoform could mean less adverse effects.

4 Minokoshi, Y. *et al.* (2002) Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415, 339–343

The Trojan horse of genetic therapy

A synthetic molecule of circular DNA has been made that can for the first time

switch off specific genes in a living cell, namely *Escherichia coli* [5]. The so-called nanocircle, developed by researchers at Stanford University (Stanford, CA, USA) and Rochester University (Rochester, NY, USA), could provide the means for developing therapies to target genes that cause cancer and other diseases such as AIDS in humans.

Circular single-stranded (ss) DNA nanocircles can be transcribed by phage and bacterial RNA polymerases (RNAP) by a method known as rolling circle transcription. By using a circular ssDNA library containing 63 nucleotides (nt) of fixed sequence and 40 nt of randomized sequence, researchers identified an ~40-nt sequence that acted as a pseudopromoter and that retained its transcriptional activity when it was transplanted to other circular DNA contexts.

Synthetic DNA nanocircles were constructed that encoded a ribozyme targeted to a particular site in the *marA* drug resistance gene of *E. coli*. By testing over 15 generations of nanocircles, the researchers located the promoter sequence that was transcribed most efficiently by *E. coli* RNAP. Once transcribed, the sequence produced ribozymes that cleaved a sequence in *marA*, causing the targeted gene to cease functioning more than 90% of the time.

Likening the nanocircle to a Trojan horse, the researchers suggest that combining synthetic nanocircle DNA vectors with optimized ssDNA promoters could be a useful way of synthesizing biologically active RNAs in living cells. Because the nanocircle vectors themselves are not replicated, the approach might be particularly helpful in situations where a drug-like approach to inhibiting gene function is most useful.

'We also would like to see if we can use nanocircles to eliminate harmful bacterium or virus by shutting down an essential gene inside the organism itself – a true Trojan horse,' commented Eric T. Kool, Professor of Chemistry at Stanford University. The team think that this nanocircle technology could also prove less expensive than making ribozymes and adding them to cells, because comparatively few nanocircles can produce thousands of ribozymes.

5 Ohmichi, T. *et al.* (2002) Efficient bacterial transcription of DNA nanocircle vectors with optimized single-stranded polymers. *Proc. Natl. Acad. Sci. U. S. A.* 99, 54–59

Designer virus has coat of many colours

By selectively modifying viral surfaces, researchers may one day be able to build the components of a molecular-scale computer or create new types of 'nanowire' with a technology that also has potential applications in medicine [6]. Scientists at The Scripps Research Institute (TSRI; La Jolla, CA, USA) and the Skaggs Institute for Chemical Biology (at TSRI) have developed a simple method for attaching a wide range of species – from small molecules to whole proteins – to the surface of cowpea mosaic virus.

The icosahedral shell of the virus is composed of 60 identical copies of a protein, each of which can be derivatized. John E. Johnson (Professor in the TSRI's Department of Molecular Biology), M.G. Finn (Associate Professor in TSRI's Department of Chemistry and The Skaggs Institute for Chemical Biology) and co-workers have successfully attached biotin, sugars, organic chemicals and proteins to the virus. Attaching short peptides containing cysteine is the key: cysteine can be easily derivatized, enabling the attachment of 'anything we want to the surface of the virus', according to Johnson. The team has attached fluorescent dyes and clusters of gold molecules to the cysteine residues, enabling them to be readily imaged. Double-labelling of the cysteines by placing cysteine both inside and outside the virus shell, creates a pattern of attachment sites that would allow for novel chemistry.

By attaching the right molecules, the stability, solubility and chromatographic properties can be fine-tuned. In addition, the virus particles can self-organize into network arrays in a crystal. The versatility of this system could lead to many applications in nanotechnology and medicine.

6 Wang, Q. *et al.* (2002) Icosahedral virus particles as addressable nanoscale building blocks. *Angew. Chem. Int. Ed. Engl.* 41, 459–462

A new rat model of ALS

A team of researchers led by Wyeth-Ayerst (Princeton, NJ, USA) and Johns Hopkins University (Baltimore, MD, USA) has developed a new transgenic rat model of amyotrophic lateral sclerosis (ALS) that could expedite the evaluation of novel treatments as well as improve understanding of the disease. Because of the larger size compared with mice, this rat model could also enable certain experiments to be carried out that have previously been difficult or impossible to carry out on ALS transgenic mice [7].

ALS, a late-onset neuromuscular disorder, results from the death of large motor neurons in the spinal cord. In ~2% of all reported cases, the condition has been linked to a mutant form of Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1), which normally converts free radical superoxide anions to hydrogen peroxide and molecular oxygen. Researchers engineered rats to carry a mutated form of SOD1 and found that the rats developed a condition similar to that in humans with ALS. Although the rats remained symptom-free for longer than traditional mice models, the disorder progressed faster than it did in the mice. This makes the changes in cells on a daily basis more striking and suggests that the rats could be useful models for testing therapeutic techniques, such as stem cell replacement.

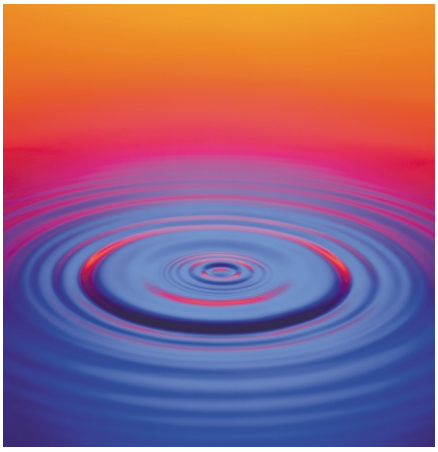
The rat models have already shown that astrocytes, cells that form >50% of the brain tissue, have a key role in the early stages of ALS. Before physical symptoms are recorded, astrocytes begin to lose EAAT2, a glutamate transporter that maintains a glutamate balance that prevents motor neurons from being overstimulated. When EAAT2 is lost, glutamate builds up, resulting, suggest the researchers, in glutamate toxicity. They hypothesize that an excess of glutamate overstimulates the neurons, which could lead to their death.

Although it is still unknown precisely how mutant SOD1 causes the death of

motor neurons or what causes EAAT2 loss, the rat models have gone some way to revealing a key step in the onset of ALS.

7 Howland, D.S. (2002) Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc. Natl. Acad. Sci. U. S. A.* 99, 1604–1609

Aquaporin regulates water movement in the lung



Researchers from the Johns Hopkins Medical Institutions (Baltimore, MA, USA) and the University of Aarhus (Aarhus, Denmark) have discovered that the aquaporin-1 protein plays a role in regulating water movement into and out of cells in the lung [8]. This insight into the molecular details of water transport in the lung could lead to new treatments for some forms of asthma, pneumonia, and pulmonary oedema or swelling.

Although the regulation of water movement is fundamental to all levels of life, the means by which water crosses cellular membranes has never been fully understood. Aquaporin proteins were first discovered some ten years ago and have been identified in many forms of life, but this is the first study to identify aquaporin-1 as a regulator of water movement in the lung.

Landon King (Assistant Professor of Pulmonary Medicine at the University of Aarhus) and Robert Brown (Associate Professor of Anesthesiology and Critical Care Medicine, Johns Hopkins) compared lung function in five people with normal levels of the protein with that of two people who lack aquaporin-1 but have otherwise normal lungs. They intravenously injected saline solution into the subjects to promote the flux of water across blood vessel walls. The size of the blood vessels increased equally in both groups, indicating that the increase in fluid volume from the infusions was the same. In marked contrast, the airway wall thickness increased 40–50% in normal individuals but was unchanged in those lacking aquaporin-1. In normal lungs, such a fluid challenge causes leakage of water through aquaporin-1 and swelling in the space around the airways. Without the protein, however, no leakage of fluid was seen.

'This suggests that water permeability is independently regulated,' said King. Passive movement across cell membranes is still very important for lung function and, in people who lack aquaporin, it could be vital, but these results show that aquaporin-1 plays a role in normal lung physiology.

King said, 'Aquaporin-1 may be a good target for developing therapies where water regulation is crucial to the functioning of the organ, including the lungs and the kidney.' The role of aquaporin-1 in the kidneys had previously been demonstrated by King, whose research will now turn to the expression, stability and movement of the protein in different species.

8 King, L.S. *et al.* (2002) Decreased pulmonary vascular permeability in aquaporin-1-null humans. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1059–1063

Clinical trials

Dosing suspended in Phase IIa clinical trial of Alzheimer's drug

Elan Corporation (Dublin, Ireland) and Wyeth-Ayerst Laboratories (St David, PA, USA) have temporarily suspended the dosing of patients in their Phase IIa clinical trials of AN1792, an experimental immunotherapeutic, after four patients in France were reported to have clinical signs consistent with inflammation of the CNS. AN1792 (also known as AIP001), a treatment for mild-to-moderate Alzheimer's disease, has been tested on ~360 patients in the US and four European countries. Some of those taken ill in France were also shown to have a viral infection of their cerebrospinal fluid, which can lead to CNS inflammation. However, the definite cause of inflammation is yet to be determined. Elan's Chief Scientific and Medical Officer, Ivan Lieberburg, said the decision was part of their standard approach to protecting patient safety.

US sets up consortium to monitor clinical trials

A Consortium to Examine Clinical Research Ethics (CECRE) has been established at Duke Medical University Center (Durham, NC, USA) to collect information on how clinical trials are overseen in medical centres and how people enrolled in such trials can be better protected. The consortium, which will contain clinical researchers, industry representatives, Institutional Review Board (IRB) members and bioethicists, comes amid criticism that some researchers have a financial interest in the outcome of their trials.

'The system to protect participants…has recently been challenged,' said Jeremy Sugarman, Director of the Center for the Study of Medical Ethics and Humanities at Duke University Medical Center. 'Concern has focused on financial conflicts of interest, the workings of the IRB and haphazard reporting of such adverse events as drug reactions,' he said.

The consortium, funded by a US$830,000 grant from the Doris Duke Charitable Foundation, will evaluate a sample of 20–25 representative research centres to compile information on the number and nature of clinical trials being conducted; the number of participants enrolled; the costs of IRB oversight and review; and the source of funding for the research.

'Although IRB review is required for federally funded research, there are no firm data on how many IRBs exist. Such a database…should provide valuable empirical information that can be used in future policy development,' he said.

The CECRE will also develop a framework for determining which research projects require heightened scrutiny and will look at efforts now used to ensure patient safety.

Clues to Down's syndrome origins

Scientists at the University of Cambridge (Cambridge, UK), University College London (London, UK) and the University of Wisconsin-Madison (WI, USA) have discovered some of the key cellular and molecular processes that give rise to Down's syndrome. The study, published in a recent issue of the *Lancet* [9], was the first of its kind to use human cells.

According to Clive N. Svendsen, a UW-Madison Professor of Anatomy and Neurology and director of the stem-cell research programme at the UW-Madison Waisman Center (a leading centre for the study of human development and neurodegenerative diseases), 'These findings point to a serious deficit in specific genes known to be important for neuronal development.'

The research determined that a neuron-specific growth-associated protein (SCG10), which is regulated by the neuron-restrictive silencer factor (REST), was almost undetectable in samples of Down's syndrome. This suggests a link between dysregulation of REST and some of the neurological deficits that characterize Down's syndrome.

Although these are preliminary results, identification of faults in the behaviour of key genes that cause Down's syndrome could lead to better treatments and even possible novel drug or gene therapies. 'Until now, we have only had mouse models…, which have not been so faithful in reproducing all aspects of Down's syndrome,' explains Svendsen. He added, 'now we have a complementary source of human stem cells with extra chromosome 21, and which can be grown indefinitely and used by a large number of scientists.' He continues to explain that this makes a 'nice model for drug…intervention to try and get (the developing brain) back to normal neuronal production from the stem cells. If we can understand the loss of neurons in Down's syndrome, I think it may lead to some novel treatments in the future.'

9 Bahn, S. *et al.* (2002) Neuronal target genes of the neuron-restrictive silencer factor in neurospheres derived from fetuses with Down's syndrome: a gene expression study. *Lancet* 359, 310–315

Miscellaneous

Three-way collaborations for breast cancer

The University of Cambridge (Cambridge, UK) is to collaborate with NextGen (Huntingdon, UK) and Cytomyx (Cambridge, UK) in a three-year research programme to develop a series of protein biochips for the analysis of breast cancer. The project, part-funded by the Department of Trade and Industry's (DTI; London, UK) LINK Programme in Applied Genetics, the Biological and Biotechnology Sciences Research Council (BBSRC; Swindon, UK) and the Medical Research Council (MRC; London, UK), will provide the University with new proteomics technologies. In turn, NextGen will develop a range of breast cancer protein biochips and a high-throughput automated protein expression Protein Library Management System (PLMS). Cytomyx will build a repository of clones for use in genomics and proteomics-based drug discovery.

'This initiative…allows us to access new technology in protein expression analysis and to combine this with the genomics and transcriptomics information we are already gathering,' said Carlos Caldas, University of Cambridge and Cancer Research Campaign researcher. 'Our aim is to make the database and bioinformatics tools we develop freely available to the research community,' he said.

A separate collaboration between Oxford GlycoSciences (OGS; Oxford, UK), Medarex (Princeton, NJ, USA) and Genmab (Copenhagen, Denmark), will lead to the creation of an array of novel medical products for breast cancer. The development effort is designed to lead to a broader panel of complementary breast cancer treatments, including new antibody and vaccine therapies, and biomarkers, that have been discovered through OGS' proteomics platform with Medarex and Genmab's combined strengths in creating and developing immunological products. The first product, a fully human therapeutic antibody that targets heparanase 1, an enzyme involved in the growth and spread of many cancers, is expected to enter clinical trials in approximately one year.

Initially seven disease targets that appear to be expressed in tumours of >85% of women with breast cancer have been chosen for further development. Medarex will then aim to create human antibody therapeutics and/or tumour vaccines based on these disease targets. The program will also aim to produce well-tolerated therapeutic intervention at multiple stages of the disease.

News in Brief was written by *Matt Brown*, *Joanne Clough*, *Joanna Owens, Ben Ramster and Linsey Stapley*

People

New President and COO for Emisphere Technologies

Emisphere Technologies (Hawthorne, NY, USA) has appointed Alan William Dunton to the new position of President and Chief Operating Officer. Dunton joins the company from The Janssen Research Foundation (JRF), which is a Johnson & Johnson company, where he was most recently President of JRF and Managing Director of Janssen Pharmaceutica. Prior to working at Janssen, Dunton was Group Vice-President of Development at the R.W. Johnson Pharmaceutical Research Institute, which is also a Johnson & Johnson company.

On the appointment, Michael M. Goldberg, Chairman and CEO of Emisphere Technologies commented that: 'Dr Dunton brings to Emisphere significant experience in pharmaceutical industry management, along with hands-on clinical development expertise…we are pleased we will be able to draw upon Alan's prior success with advancing research programs to successful commercial products.'

1359-6446/02/$ – see front matter PII:S1359-6446(02)02223-7