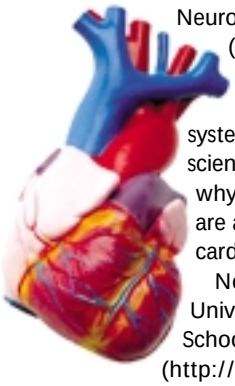
News in brief

Targets and mechanisms

Hearty new role for *Nf1* gene



Neurofibromatosis type 1 (NF1) is primarily known to cause tumours of the nervous system. However, until now scientists did not understand why patients with NF1 are also susceptible to cardiovascular problems.

Now, researchers at the University of Pennsylvania School of Medicine (http://www.med.upenn.edu) have shown how the *Nf1* gene is essential in endothelial cells – those that constitute blood vessels [1].

Mutations in the *Nf1* gene cause NF1, which occurs in 1 in every 4000 births, and researchers are hoping these findings could lead to new therapeutics for this disorder, also known as Recklinghausen neurofibromatosis.

Jonathan A. Epstein, Assistant Professor in the Cardiovascular Division of Penn's Department of Medicine, said: 'We've known NF1 as primarily a problem among cells of the developing neural crest – the part of the embryo that forms the peripheral nervous system.' He continued, 'NF1, however, is associated with cardiovascular problems, which our findings could explain by linking the loss of the *Nf1* gene to abnormal function of endothelial cells.'

The *Nf1* gene encodes neurofibromin, which suppresses the *ras* oncogene, thereby acting as a tumour suppressor. In endothelial cells, *ras* oncogenes also overactivates the NFATc1 protein, related to heart valve development.

The team found that when the *Nf1* gene was deactivated in the neural crest, tumours develop but there were no cardiac problems. Conversely, the heart develops normally regardless of *Nf1* function in the neural crest.

'The more we learn about the mechanisms behind [NF1], the better our options for treating the disease,' said Epstein. He continued, '…understanding how the gene works provides opportunities for therapies that will need to be tested.'

1 Gitler, A.D. *et al.* (2003) Nf1 has an essential role in endothelial cells. *Nat. Genet.* 33, 75–79

Genes, molecular triggers and heart failure

The previously unknown molecular sensory machinery of heart muscle cells (cardiomyocytes) has been determined by researchers at the University of California, San Diego, School of Medicine (http://medicine.ucsd.edu) [2]. Normally, when stimulated by mechanical stretching, the stretch sensory complex in cardiomyocytes triggers cell growth and survival. Defects in the complex are linked with an inherited form of heart failure [dilated cardiomyopathy (DCM)], in which an enlarged heart loses its ability to pump blood.

Defects in a portion – the Z-disc complex – of the heart's muscular scaffold can cause the heart to lose the ability to adapt to the stress of increased mechanical stretch that occurs during each heartbeat. When mutated in both mice and humans, a gene called muscle-specific LIM protein (MLP), which is located in the elastic Z-disc complex, causes misalignment of molecular components called titin and telethonin [(T-cap), a titin interacting protein]. The mutant Z-disc MLP/T-cap complex is unable to sense the stretch stimulus necessary for triggering the growth and survival signals.

Mutation in MLP is responsible for a significant proportion (10%) of inherited DCM sufferers in Northern Europe, making it the single largest mutation linked to this form of human heart failure. Evidence also points to the MLP mutation as being a founder effect, that is, a common distant ancestor responsible for the gene defect.

Mice deficient in heart protein phospholamban (PLN) exhibit no such stretch-sensor defects and retain normal-sized hearts. Co-author Masahiko Hoshijima believes that 'the MLP deficiency could potentially be corrected with gene therapy using a modified form of PLN'. This clarification of the molecular basis of DCM will hopefully lead to novel molecular therapies for the disease.

2 Knöll, R. *et al.* (2002) The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 111, 943–955

Anti-asthma researchers target Ref1

Scientists have designed a novel small molecule that can alleviate the symptoms of asthma. Groups based at the Pacific Northwest Research Institute (http://www.pnri.org/) and the University of Washington (http://www.washington.edu/) used a powerful chemogenomics approach to develop a factor that successfully reduces swelling of airways in a mouse model of the disease [3].

Characterization of their designer molecule showed it to inhibit the redox-effector factor Ref1, suggesting that Ref1 could become an important future target for anti-asthma drugs.

Asthma affects ~200 million people worldwide. The airway inflammation that characterizes the disease is known to involve activation of the redox-sensitive transcription factors activator-protein 1 (AP1) and nuclear-factor κB (NFκB).

Michael Kahn and co-workers used a combinatorial library approach to develop an array of related small molecules that were potential inhibitors for AP1 and NFκB. They then screened the products for their ability to affect transcription of AP1- and NFκB-reporter constructs in human lung epithelial cells. The compound PNRI299 selectively inhibited transcription of the AP1-driven reporter, and affinity chromatography revealed that PNRI299 interacts specifically with Ref1, which normally activates AP1. Kahn and colleagues went on to test PNRI229 in a mouse model of asthma and demonstrated that it limited mucus production and invasion of the lung lining by eosinophils, monocytes and macrophages, and decreased expression of the Ref1-activated pro-inflammatory factor, interleukin-4.

The researchers on this is study have made significant progress towards production of an effective anti-asthma treatment. They have also identified Ref1 as a potential therapeutic target and their chemogenomic approach is likely to form the basis of future studies in the fight against asthma.

3 Nguyen, C. *et al.* (2003) Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1169–1173

1359-6446/03/$ – see front matter PII: S1359-6446(03)02637-0

Miscellaneous

Way to glow!

The glow of fireflies could shed light into how well new drugs work, showing whether they are killing cells or causing other effects [4].

Scientists at the University of Michigan Health System (http://www.med.umich.edu) report that they have inserted the gene for the molecule that produces the glow of a firefly into mice with cancer [3]. They kept the molecule from producing light until the cells began to die in response to cancer treatment.

The faintest traces of light can be detected using a highly sensitive camera; this technique could provide a new way of obtaining real-time information on the effectiveness of medicines and could be used to monitor cellular processes.

Alnawaz Rehemtulla, Assistant Professor of Radiation Oncology at the U-M Medical School and lead author of the paper, said: 'This is the first time anyone has been able to make real-time images of apoptosis… This proves that we can see what's going on at the molecular level while the drugs are working, giving results in days or weeks instead of months or years.'

The enzyme that triggers a chemical reaction that causes the glow is luciferase, which emits bioluminescence and has been used in biomedical research for several years in an 'on' state: the team in this study found how to turn it off by attaching it to a portion of the estrogen receptor (ER), which quenches the action of luciferase. A 'switch' was added to turn the enzyme on when apoptosis occurs.

This 'light switch' was developed by inserting a small section of protein (DEVD) between the luciferase and ER: DEVD is a target of caspase-3, one of the key enzymes that activates apoptosis. Caspase-3 is most active when cells are dying, and so when apoptosis occurs, caspase-3 cleaves DEVD and enables luciferase to be expressed, which is detected as luminescence.

The ability to image apoptosis non-invasively will enable screening for compounds that regulate apoptosis and could be an invaluable tool for in target validation *in vivo*.

4 Laxman, B. *et al.* (2002) Noninvasive real-time imaging of apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 99, 16551–16555

More effective heparin

Systematic studies on the formulation and structure of heparin, and the enzymes that degrade it, could lead to significant improvements in thrombosis prevention [5]. One such application, now entering Phase III trials, could benefit the 600,000 patients undergoing a coronary artery bypass graft each year.

Heparin and the related low-molecular-weight heparin (LMWH) are drugs commonly used in surgery to prevent blood clots. However, heparins are of highly variable structure and their efficacy can vary from dose to dose. Ram Sasisekharan of MIT's Biological Engineering Division (http://web.mit.edu/be/) has been studying this problem since the early 1990s. Previously, he successfully cloned the heparinase gene, which codes for an enzyme that breaks down heparin. Heparinase was subsequently developed as a tool for determining the order of carbohydrate groups in complex sugars such as heparin.

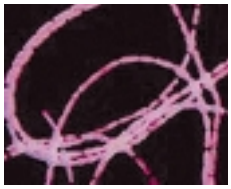
Using this technology, Sasisekharan and co-workers were able to probe and manipulate the structure of heparin's active sites and identify the motifs associated with anticoagulant activity. The work has now reached the stage where the group can redesign heparins. Recently, they made two forms of LMWH with specially engineered activity profiles.

In rats, the new formulations were much more potent than conventional forms of the drug. In addition, the tailor-made heparins were neutralized by the body after a suitable interval. This capability is a big advantage over the conventional drug, which must be inactivated by administration of protamine after surgery. Protamine is a non-specific inhibitor and causes side effects, so an alternative method has long been sought.

As well as being used to tailor heparin drugs, heparinases are also promising drugs in their own right. In conjunction with BioMarin Pharmaceuticals (http://www.biomarinpharm.com/), Phase III clinical trials have recently been approved to test heparinase I as a protamine substitute.

5 Sundaram, M. *et al.* (2003) Rational design of low-molecular weight heparins with improved *in vivo* activity. *Proc. Natl. Acad. Sci. U. S. A.* 100, 651–656

A beneficial use for anthrax?



Scientists based at the National Institutes of Health (http://www.nih.gov/) have revealed a beneficial use for anthrax – to kill tumour cells *in vivo* [6]. Stephen Leppla and colleagues have engineered the anthrax toxin protective antigen and demonstrated that it effectively killed several types of tumour cells in mice and had no apparent toxic effect on normal tissue.

Malignant cells are characterized by a high level of urokinase protein as a result of cell-surface urokinase plasminogen activator activity. Hence, this protein represents an attractive target for anticancer therapy. In the engineered version of the anthrax toxin protective antigen, the furin activation sequence was replaced by a synthetic peptide that makes the tumouricidal activity of this toxin dependent on cell-surface urokinase.

After only one treatment with the engineered anthrax toxin, the size of tumour cells was reduced by 65–92% in mice and, after two treatments, up to 88% of fibrosarcinomas and 17% of melanomas were killed. The tumour cells started to die within 12 hours after the first treatment and there was no apparent damage to surrounding skin cells or hair follicles, suggesting that the toxin is specific to tumour cells.

Anticancer treatments in humans are associated with severe side effects, and the prospects of using an engineered anthrax toxin with specific tumouricidal activity and limited toxicity as a basis for anticancer therapy is encouraging news. The next step is to see if the engineered anthrax toxin will have similar effects in humans.

6 Liu, S. *et al.* (2003) Potent antitumour activity of a urokinase-activated engineered anthrax toxin *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0236849100 (http://www.pnas.org/)

Algae-tastic news for complex antibody production!

A highly effective method for large-scale production of complex proteins, such as antibodies, has been hit upon by a team at The Scripps Research Institute (TSRI; http://www.scripps.edu) [7]. The highly innovative procedure, using algae as the

host, could transform the production of complex human antibodies – and, indeed, other potential therapeutic proteins – from current low levels at high cost to massive scale at little expense.

In this case, researchers at TSRI – led by Associate Professor Stephen Mayfield –used the unicellular, green algae, *Chlamydomonas reinhardtii*, to synthesize a unique large single-chain (lsc) antibody that targets the herpes simplex virus. The gene for lsc is inserted into the genome of the chloroplast, which, unlike bacteria (commonly used to produce an abundance of simple proteins), contains all the machinery that is necessary to assemble complicated proteins. Expression of the chimeric gene is driven by either of two *C. reinhardtii* chloroplast promoters and 5′ and 3′ RNA elements. The lsc antibody is produced in a soluble form, assembles into higher order complexes *in vivo*, and undergoes no obvious posttranslational modification.

Antibodies can be useful as therapeutics for many other human diseases, including leukemia. Mayfield said, 'We think we can now put in pretty much any gene that we want and have it expressed,' and thousands of litres of the algae can be grown once they are modified. Thus, a whole new therapeutics doorway has been opened.

7 Mayfield, S.P. *et al.* (2003) Expression and assembly of a fully active antibody in algae. *Proc. Natl. Acad.Sci. U. S. A.* 100, 438–442

ACE crystal structure

The 3D structure of an enzyme that is implicated in several cardiovascular diseases has been solved [8]. Researchers at AngioDesign (http://www.angiodesign.com/) have elucidated the X-ray crystallographic structure of angiotensin-converting enzyme (ACE). They now hope to use the structure to develop selective and safe next-generation drugs.

ACE, a two-domain enzyme that is present in the blood plasma, activates the hormone angiotensin, which causes vasoconstriction. Blocking the enzyme has therefore proven to be an effective way of treating various cardiovascular diseases by lowering blood pressure. ACE inhibitors have found application in diseases such as hypertension, heart failure, coronary heart disease and kidney failure.

However, most ACE inhibitors were developed, in the 1970s and 1980s, without knowledge of the enzyme's structure, and commonly cause side effects such as mild coughing. The enzyme has two domains, which perform different functions. Current inhibitors are non-selective and block both domains, leading to the side effects. It is hoped that the solution of the crystal structure of ACE, in complex with the inhibitor lisinopril, will enable the design of safer and more selective drugs.

The team at AngioDesign intend to use their results for structure-guided drug design, with the enzyme–lisinopril complex as a starting point for optimization.

8 Natesh, R. *et al.* (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature*, 421, 551–554

Inheriting RNAi

The power of RNA interference (RNAi) has been extended, enabling the silencing of gene expression to be inherited in mice. The technique was developed by researchers at Stony Brook University (http://www.sunysb.edu/) and Cold Spring Harbor Laboratory (http://www.cshl.org/), and can be used as a complement to standard knockout methodologies in the study and treatment of many human diseases [9].

RNAi is a technique in which short hairpin sections of single-stranded RNA block cellular RNA by complementary binding. In a new application of the technology, the researchers genetically engineered mouse embryonic stem cells with RNAi targeted to the novel *Neil1* gene, which is implicated in DNA repair.

These stem cells were injected into mouse embryos, and the chimeric offspring were crossed to give mice whose cells all contained the RNAi-inducing gene. Expression of the gene was reduced by around 80% in all tissues, a phenomenon known as gene knockdown. The cells also showed a twofold increase in sensitivity to ionizing radiation, consistent with the proposed function of the gene product.

In future, the work could be adapted to selectively reduce or enhance expression in specific tissues. Such a genetic switch could have many applications in the treatment of disease.

9 Carmell, M.A. *et al.* (2003) Germline transmission of RNAi in mice. *Nat. Struct. Biol.* 10, 91–92

Cancer Targets and Mechanisms

A new gene target for breast cancer

Recent research has identified a possible new gene target for use in the diagnosis or treatment of breast cancer. The gene is expressed in many breast cancers but is not found in other normal tissues, except the salivary glands [10].

The team of researchers, led by Ira Pastan at the National Cancer Institute (http://www.nci.nih.gov/), screened RNA molecules that are overproduced by breast and prostate cancer cells, and found >3000 new RNA transcripts that are currently unrelated to any known genes. They then investigated experimentally the expression of one of those transcripts that encodes a protein that they designated BASE (breast cancer and salivary gland expression). They found that *BASE* is not expressed in normal tissues but that it is expressed in many breast cancers. Although the function of the gene is not yet known, it is thought to be secreted from the cell.

The researchers hope that, by analyzing the RNA library further, they might find additional gene products that could also be used in the treatment and diagnosis of breast and prostate cancers, perhaps leading to an eventual vaccine target.

10 Egland, K.A. *et al.* (2003) Discovery of the breast cancer gene *BASE* using a molecular approach to enrich for genes encoding membrane and secreted proteins. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1099–1104

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