lacks the negative charges that are present along the DNA backbone.

Currently, they are working to characterize the hybridization of short PNAs to G-rich DNA. 'As we learn more about the range of quadruplexes that PNA can form, then we can start to think rationally about targeting actual biological systems,' Armitage said.

### Therapeutic approaches

There is evidence that a G-quadruplex, which forms upstream of the c-Myc gene within chromosomal DNA, must be unfolded in order for a transcription factor to bind and activate gene expression [4]. 'If you have something that's bound very tightly to the guanine-rich sequence, then it could prevent expression of the oncogene,' Armitage said. It is possible that a homologous PNA could either hybridize with the G-rich strand to form a quadruplex or to the C-rich strand to form a duplex – both of which would theoretically block transcription factor binding and c-Myc expression.

PNAs have many features, including high DNA and/or RNA-binding affinity, recognition specificity, biochemical stability and remarkable strand-invasion ability, that suggest their potential as therapeutics. However, work to date, including the current study, has occurred in a simplified model system. 'Hence, sequence and structure specificity of quadruplex-forming PNA ligands, and workability of their isothermal binding to corresponding nucleic acid targets at physiological temperatures, have to be thoroughly addressed for the use of these G-rich PNAs in biologically relevant complex systems,' said Demidov. 'Some more difficulties, such as poor solubility, target accessibility and tissue-specific or intracellular delivery, could also be encountered.'

### References


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2 Nielsen, P.E. *et. al.* (1991) Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* 254, 1497–1500

3 Smith, F.W. and Feigon, J. (1992) Quadruplex structure of telomeric DNA oligonucleotides. *Nature* 356, 164–168

4 Siddiqui-Jain, A. *et al.* (2002) Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11593–11598


# News in brief

## Targets and Mechanisms

### Multi-talented enzyme

The structure of an enzyme that is key to the initiation of many biochemical pathways has been elucidated by researchers at St Jude Children's research hospital (http://www.stjude.org) [1]. A group led by Brenda Schulman revealed that the enzyme uses two different parts of its own structure to juggle four different molecules as it completes three different reactions.

Vital cell processes are switched on by ubiquitin-like proteins such as NEDD8, which are activated by E1 enzymes to co-ordinate specific functions and ensure that they take place at the right time. The activation of ubiquitin-like proteins is actually a complex series of reactions that begins when a specific E1-activating enzyme brings together a ubiquitin-like protein and an E2 escort molecule. E2 escorts the ubiquitin-like protein to its pre-assigned target molecule for chemical modification, triggering a specific cellular activity.

Structural characterization of the E1 enzyme for NEDD8 has helped to explain how E1 manages to complete the three different reactions required to link NEDD8 to its E2 escort – they occur at distinct regions of the enzyme.

It is thought that this knowledge could help to explain the complex command and control systems that are disrupted in disease. For example, the influenza virus hijacks a ubiquitin-like protein so that it cannot undergo normal activation by an E1 enzyme. This helps the virus to hide from the immune system. 'The more we learn about how these pathways are controlled, the more likely it is that we'll understand how to fix them when they're disrupted,' stated Schulman.


1 Walden, H. *et al.* (2003) Insights into the ubiquitin transfer cascade from the structure of the activating enzyme for NEDD8. *Nature* 422, 330–334


### AD and PD: a new link

The two most common neurodegenerative diseases have been linked at the molecular level. Researchers at the University of Pennsylvania School of Medicine (http://www.med.upenn.edu/) have discovered that a protein implicated in Parkinson's disease (PD) can induce the aggregation of a protein associated with Alzheimer's disease (AD) [2]. This new connection could explain why patients with one disease are more likely to show signs of the other.

Both PD and AD are characterized by amyloid lesions caused by clumps of tangled proteins. The protein α-synuclein is one such contributory factor in PD, in which it binds to itself enabling the formation of Lewy bodies. The tau protein has a similar role in the onset of AD, but is larger and requires cofactors to initiate the formation of fibrous clumps. Both proteins are naturally abundant in the brain, where they have distinct functions: tau has a role in microtubule stabilization, whereas α-synuclein is involved in regulating communications at the synapse.

Beginning with *in vitro* studies, the researchers found that α-synuclein interacted

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with tau, causing it to fold and aggregate. Extending to an *in vivo* setting, they next discovered that α-synuclein polymerization alone is sufficient to induce the aggregation of tau clumps in mouse brain. Lead author Benoit Gaisson explained: 'Tau and α-synuclein work together to promote and propagate each other's formation of fibrous clumps and, hence, the amyloid lesions that cause disease.' This synergistic relationship could be leveraged to design therapeutics that could be efficacious against several neurodegenerative diseases.

2 Giasson, B.I. et al. (2003) Initiation and synergistic fibrillization of tau and α-synuclein. *Science* 300, 636–640

### APC's the great pretender

Tiny magnetic beads have been turned into artificial antigen presenting cells (aAPCs) enabling the large-scale production of targeted cytotoxic lymphocytes (CTLs) against cancer and viral infections [3]. This development could overcome the main problem with current production methods for targeted immune cells – the variation in number and quality of a patient's own APCs (dendritic cells).

APCs convert generic immune cells into targeted CTLs by displaying antigens from cancer cells or viruses on their surface, priming CTLs to recognize these antigens and kill the foreign cells. Researchers from Johns Hopkins University School of Medicine (http://www.hopkinsmedicine.org/medicalschool/) have managed to create twice as many specific, targeted CTLs using aAPCs compared with natural APCs.

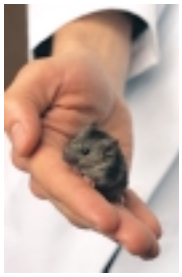
Mathias Oelke and colleagues coated magnetic beads with soluble human leukocyte antigen-immunoglobulin fusion protein (HLA-Ig), which mimics the antigen-presenting activity of natural APCs [4], and CD28-specific antibody. The beads were then exposed to melanoma or cytomegalovirus (CMV) antigens and used to induce and expand specific CTLs. Cultures induced with aAPCs increased the number of specific CTLs in successive rounds of stimulation, resulting in the generation of a clinically relevant population of CTLs that recognized endogenous antigen–MHC complexes present on melanoma cells.

Jonathan Schneck, senior author on the paper, commented: 'The ability to make vast quantities of targeted, antigen-specific immune cells in the lab broadens their potential in tackling a wide array of diseases, especially cancers. Our technique provides an off-the-shelf way to create these cells.' Before testing in patients, however, the production method must be modified to produce clinical grade cells, a process that could take from two to four years, according to Oelke.

3 Oelke, M. *et al.* (2003) Ex vivo induction and expansion of antigen-specific cytotoxic T cells by HLA-Ig-coated artificial antigen-presenting cells. *Nat. Med.* 9, 619–625
4 Hamad, A.R. *et al.* (1998) Potent T cell activation with dimeric peptide-major histocompatibility complex class II ligand: the role of CD4 coreceptor. *J. Exp. Med.* 188, 1633–1640

## Cancer Targets and Mechanisms

### Cancer fighting mice



Spontaneous regression of cancer is rare and poorly understood. The recent discovery of mice in which cancer regression is the norm offers an important insight into this phenomenon and provides a valuable new tool for cancer researchers [5].

Zheng Cui and colleagues at Wake Forest University School of Medicine (http://www.wfubmc.edu/school/) made their almost serendipitous discovery when injecting mice with cells that form highly aggressive cancers in all strains of laboratory mice. Surprisingly, one mouse remained cancer-free, despite repeated injections. Breeding revealed that the anti-cancer trait is linked to a single locus and is inherited dominantly. Cui and colleagues were able to establish colonies of the protected mice, many of which were completely resistant to injections of cancer-causing cells. Of the mice in which cancer could begin to develop, many were free of tumour cells 24 hours later and subsequently had complete resistance. However, susceptibility to cancer was greater in older mice, reminiscent of the increased incidence of the disease with age in humans.

The researchers also investigated the mechanism behind the protection. They found that injection of cancer cells into the resistant mice provoked a massive infiltration by host leukocytes. These cells formed aggregates around the tumour cells and destroyed them rapidly, apparently without damage to normal cells. 'These observations suggest a previously unrecognised mechanism by which the body can fight off cancer', said Cui, Associate Professor of Pathology at Wake Forest.

The discovery of mice with innate protection from cancer provides a unique opportunity for researchers to study, and perhaps to harness, the mechanisms of spontaneous regression. 'This is at a preliminary stage, but very promising' says Mark Willingham, another of the researchers. 'Our hope is that, some day, this will have an impact on human cancer.'

5 Cui, Z. *et al*. (2003) Spontaneous regression of advanced cancer: identification of a unique genetically determined, age-dependent trait in mice. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1031601100

### Cell cycle guard in cancer conspiracy!

A new factor that is likely to be involved in aggressive tumour growth has been discovered by researchers at the Human Cancer Genetics Program at Ohio State University's Comprehensive Cancer Center (http://www.osumedcenter.edu/). When inactivated in mouse embryo fibroblasts, transcription factor E2F3 causes centrosome duplication, creating mutant daughter cells, which – if they survive – show increased unchecked growth [6].

E2F3 regulates centrosome duplication cycles, ensuring that exact copies of a parent cell's chromosomes are transmitted to daughter cells. In most cases of E2F3 deficiency, the resulting genetic instability slows cell division. However, the reverse can happen in some instances, leading to increased cell division potential for mutant cells; also, depending on the area of the body, these cells can spread throughout the body. However, Gustave Leone, who led the study, said that 'the loss of E2F3 itself does not cause cancer, but its absence gives a normal cell the chance to change into a cancer cell'.

Normally, cells undergoing division have two centrosomes, but the E2F3-deficient mouse cells in culture have greater than two centrosomes, and sometimes more than four. Similarly, cells in many human tumours have too many chromosomes, which might be one of the key factors in the development of breast, prostate and colon tumours.

Leone also stated: 'If it turns out that a lack of E2F3 triggers the onset of a tumour

by causing excessive centrosomes in a cell, then there may be some way we could prevent centrosome duplication.' To test the E2F3–cancer connection more thoroughly, mice with tumours will need to be analyzed to identify whether centrosome duplication occurs before the tumour actually begins to grow.

6 Saavedra, H.I. *et al*. (2003) Inactivation of E2F3 results in centrosome amplification. *Cancer Cell* 3, 333–346

## Miscellaneous

### An explosive new vaccine vehicle

A novel vaccination method that could have many other applications has been developed using bespoke polymer beads [9]. The tiny polymer spheres, developed by a team from the University of California, Berkeley (http://www.berkeley.edu/) and the Lawrence Berkeley National Laboratory (http://www.lbl.gov/), have several advantages over more traditional methods as a delivery vector.

Many diseases, such as AIDS, cancer and hepatitis, are in urgent need of reliable vaccination strategies. The traditional approach of inoculation through the introduction of attenuated virus is often unsuccessful and can be toxic. In recent years, attention has turned to protein-based vaccines that seek to overcome such limitations. One of the main challenges in this area is the design of a suitable delivery vehicle. Polymer bead technologies are a hotly tipped approach, whereby the protein is encapsulated in a polymeric vector, which then enters an immune system cell and activates the wider immune response.

The technique has been of limited success, however, mainly because of difficulties in persuading antigen-presenting cells (APCs) to display the antigen on their surfaces to trigger an immune response that primes the body against further encounters with the antigen. The latest microgel polymer-bead-technology shrewdly avoids this problem. Once an APC engulfs the polymer bead, it is exposed to the acidic conditions of the cell's digestive phagosomes. These pockets would normally break down the proteins, preventing their presentation to the wider immune system on the cell surface. Crucially, however, the beads are designed to break apart under acidic conditions. In the phagosome, they fall apart into thousands of molecules, drawing in water from the cytoplasm by osmosis. This causes the phagosome to burst, spewing the antigen protein into the cytoplasm and enabling it to be expressed on the cell surface. The technique was successfully demonstrated in cultured cells and is now being tested in mice.

The acidity at which the beads break down can be tailored, enabling the bead to be selective for the conditions of the phagosome. The construction technique can also be modified to create beads of different sizes, enabling the technnology to be adapted to areas such as gene delivery. This gives the system a generality that excites lead researcher Jean Fréchet, Head of Materials Synthesis in the Materials Science division at Lawrence Berkeley: 'There are much flashier methods that are more complicated but probably not practical,' he commented.

## Genomics and Proteomics

### New genomes: guts and glory

Two bacteria found in the human gut have had their genomes sequenced – and for very different reasons. The genome of *Bacteroides thetaiotamicron* was decoded as a first step in the 'next frontier' of microbiome research; that is, the genetic analysis of the many symbiotic bacteria that subsist within humans. In a second development, the genome of *Enterococcus faecalis* should help researchers investigating antibiotic resistance acquired by gene transfer in bacteria.

To gain an insight into the rich environment of the microbiome, a team headed by Jeffrey Gordon of the Washington School of Medicine (http://www.washington.edu) sequenced the genome of *B. thetaiotamicron*, one of the most prevalent bacteria in the human gut [7]. The species becomes prevalent in the infant human's intestines at the time the child is weaned, reflecting its role in digesting complex polysaccharides, which enter the human diet at this stage.

The genome revealed some of the strategies the microbe uses to coexist with its host. Around 5% of its genes code for proteins that retrieve dietary polysaccharides from the intestine. These enzymes break down the polysaccharides into simpler molecules that can then be absorbed by both bacteria and host. The bacterium also devotes a small chunk of its genome to genes that allow the manufacture of different carbohydrates on the cell surface, which probably assist evasion of the host's immune system. In addition, a highly elaborate system for detecting carbohydrate concentrations was discovered. As well as providing a vital insight into the co-evolution of bacteria and humans, detailed knowledge of the microbiome could lead to the discovery of natural products – potential leads in the search for new drugs.

In a related development, researchers at The Institute for Genomic Research (http://www.tigr.org/) sequenced the genome of the gut bacteria *E. faecalis* V583 [8]. This bacterium can cause infections when introduced to tissues other than those of the gastrointestinal tract, and is often passed on by fecal contamination. Of particular concern is the resistance of the V583 strain to vancomycin, a drug that is only used when all others have failed. Indeed, the strain can act as a 'reservoir', passing on resistance to other, more dangerous types of bacteria. For this reason, *E. faecalis* was high on the priority list of bacteria to be sequenced.

Genome analysis found that nearly a third of the bacterial DNA comprises mobile genetic elements such as plasmids and transposons that can jump between organisms. This unusually high proportion, including two sites that confer resistance to vancomycin, explains how *E. faecalis* can acquire – and pass on – resistance to drugs.

7 Xu, J. *et al.* (2003) A genomic view of the human-bacteroides thetaiotaomicron symbiosis. *Science* 299, 2074–2076

8 Paulsen, I.T. *et al.* (2003) Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. *Science* 299, 2071–2074

9 Murthy, N. et al. (2003) Inaugural article: a macromolecular delivery vehicle for protein-based vaccines: acid-degradable protein-loaded microgels. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4995–5000

## Modelling influenza

A new theoretical model that shows how the pressure exerted by the immune response of an infected population can drive evolution of influenza virus has recently been developed [10]. It is hoped that this general approach to modelling could also be used to understand the evolution of other RNA viruses, including HIV.

The model does not aim to predict the emergence of new strains of influenza, however, it does suggest that a short-lived general immunity to the virus might affect the evolution of the virusand could aid vaccine development.

To explore evolutionary dynamics, Neil M. Ferguson and colleagues at Imperial College, London (http://www.ic.ac.uk) developed a computer-intensive mathematical model that stimulated mutation in individual codons of the viral coat and the effect of those changes on the transmission of the virus in human populations. Their hypothesis was that modelling could yield information on the genetic diversity of the virus population that would result from changes induced by mutation.

'The best fit to genetic data was obtained when a secondary, non-specific immune response was included in the model, on top of the normal adaptive immune response, which recognizes individual virus strains.' says Ferguson.

Virologists previously thought that temporary, non-specific immunity might exist but not as a significant driver of influenza evolution or epidemiology. This new work, however, suggests that both influenza transmission and evolution are crucially affected by non-specific responses. Because the mechanism remains unknown, Ferguson adds that it is yet to be seen whether it might provide the basis of a more general influenza vaccine.

10 Ferguson, N.M. *et al.* (2003) Ecological and immunological determinants of influenza evolution. *Nature* 422, 428–433

## Better heart drug with NO extra

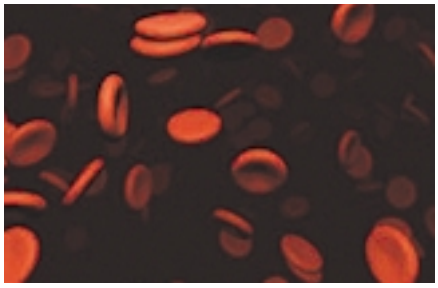
A chemical relative of nitric oxide (NO) has been shown to restore function in failing hearts without affecting the heart's ability to relax and pump effectively [11]. Although NO-releasing medicines are widely used and are able to effectively increase the heart's ability to contract, they also blunt the signals that enable the heart to fully relax and pump blood efficiently.

David A. Kass and colleagues at the Johns Hopkins Medical Institutions (http://www.jhmi.edu/) tested the effects of an infusion of Angelis salt, which generates a negatively charged form of NO called nitroxyl anion ($NO^-$), on dogs with and without heart failure. $NO^-$ doubled the heart's ability to contract and, unlike NO, enhanced the ability of the heart to relax, both in failing and normal hearts. Furthermore, it stimulated the release of calcitonin gene-related peptide (CGRP), which dilates blood vessels, and greatly enhanced the influence of another stimulator of heart function, dobutamine.

'This is a truly novel pathway for stabilizing a failing heart,' said Kass, Professor of Medicine and Biomedical Engineering at Johns Hopkins, '[and] could prove to be a useful alternative to traditional NO treatments that only enhance part of the pumping cycle.'

11 Paolocci, N. *et al.* (2003) Positive inotropic and lusitropic effects of $HNO/NO^-$ in failing hearts: independence from beta-adrenergic signaling. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5537–5542

## Blood cells not left Wnting



Two papers have reported the role of Wnt proteins in activation and renewal of cells in the haematopoietic system, as well as the first successful attempt to purify Wnt in an active form [12,13]. Wnt proteins are well known to be involved in developmental signalling, but until now efforts to purify and characterize this important class of cell factors has been hampered by their high degree of insolubility.

A collaboration between Roel Nusse's group at Howard Hughes Medical Institute (http://www.hhmi.org/), Karl Willert's group at Stanford University School of Medicine (http://www.med.stanford.edu/) and Irving Weissman's laboratory at Duke University, North Carolina (http://www.duke.edu/) revealed the reason for this high insolubility – the proteins have a palmitoyl group attached to a conserved cysteine within their amino acid sequence. Enzymatic removal of the palmitoyl group resulted in loss of activity of the Wnt protein, which suggests this lipid modification has a role in Wnt signalling [12].

Further investigation into the product of the mouse Wnt3a gene showed that Wnt induces self-renewal of haematopoietic stem cells (HSCs) [12,13]. Specifically, the overexpression of beta-catenin, a co-activator of Wnt proteins, was shown to expand a pool of HSCs in long-term cultures, while maintaining the stem cells in their immature state. In another experiment, the ectopic expression of axin or a frizzled ligand-binding domain – both inhibitors of the Wnt pathway – resulted in inhibition of HSC growth in vitro and reduced reconstitution in vivo. Finally, activation of Wnt was shown to increase the expression of genes involved in HSC renewal, namely HoxB4 and Notch [13].

These findings have major implications for stem cell therapy in cancer patients who are left immunocompromised after chemotherapy. Even though stem cells can be isolated, inducing them to proliferate for use in treatment has remained challenging. 'With these studies, we can now imagine isolating and expanding a patient's stem cells using activated Wnt proteins before they are treated with chemotherapy which destroys their immune system', said Nusse. 'These proliferated cells could then provide a powerful way to restore the haematopoietic system.'

12 Willert, K. *et al.* (2003) Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 10.1038/nature01611 (epub ahead of print; http://www.nature.com)
13 Reya, T. *et al.* (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 10.1038/nature01593 (epub ahead of print; http://www.nature.com)

News in brief was written by *Matt Brown, Joanna Owens, Clare Rathbone* and *Heather Yeomans*