is to couple the B-subunit to Brewer's metallic supramolecule.

'This is the next stage and it should take us a few months to work out the chemistry,' says Brewer. The process, she says, will involve adding an amine or carboxylic acid to her molecule and then coupling it to the B-subunit. 'We are also going to see how this supramolecule works alone,' she says. The molecule could also be used as an antimicrobial.

After testing the system on cells they then plan animal studies to optimize different laser parameters. Using multiple light wavelengths and pulsing techniques, Meissner says he will then work out how to best concentrate light on malignant cells.

'There are many different parameters, such as molecule concentration, efficiency of the photochemicals and the amount of light you can concentrate on the tumour,' Meissner adds.

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News in brief

Targets and mechanisms

Schizophrenia and bipolar disorder linked by a DRIP

New insight into the etiology of schizophrenia and bipolar disorder has been provided by a recent study looking at the levels of neuronal calcium sensor-1 (NCS-1) in the prefrontal cortex of patients with these conditions [1]. Abnormalities in the dopamine system have been postulated for both schizophrenia and bipolar disorder, although the exact sites of alterations remain elusive. However, the recent discovery of multiple receptor-interacting proteins (DRIPs) and their role in modifying and expanding the functionality of these receptors, has led to the suggestion that there could be altered levels of certain DRIPs in specific areas of schizophrenic and bipolar brains.

NCS-1 is present in neuronal cells throughout the brain and is a member of the DRIP family. It is present in high levels in the human prefrontal cortex and can form complexes with G-protein-coupled receptor kinase-2 and D2 dopamine receptor, making it a suspect molecule in schizophrenic and bipolar patients. In this study, 50% higher levels of NCS-1 were found in schizophrenic and bipolar patients than in controls and in patients with depression.

These findings support the hypothesis that abnormalities in dopamine receptor-interacting proteins (e.g. DRIPs), and now altered levels of NCS-1, could be associated with schizophrenia and bipolar disorder. Although schizophrenia and bipolar disorder might be induced by different factors, they could share the same mechanisms that cause the observed abnormal brain function. This study, therefore, has important implications for the treatment of these conditions.

1 Ok Koh, P. *et al.* (2003) Up-regulation of neuronal calcium sensore-1 (NCS-1) in the prefrontal cortex of schizophrenic and bipolar patients. *Proc. Natl Acad. Sci. U. S. A.* 100, 313–317

The proteasome: it's just so degrading!

The process of degrading proteins no longer needed by cells is essential in the normal growth, development and regulation of cells. Scientists at UT Southwestern Medical Center at Dallas (http://www3.utsouthwestern.edu/) have identified a new and surprising mechanism by which a class of enzymes responsible for the breakdown of proteins operates [2]. These findings have implications for understanding diseases such as Parkinson's and several forms of cancer.

Many diseases involve the inappropriate accumulation of unneeded or damaged proteins, and cells normally use an enzyme called the proteasome to remove these proteins by cutting them into small pieces. The proteasome, which is present in all higher cells, contains its active sites inside a cylinder-like shape with a gate that prevents the entry of normal cellular proteins, thereby protecting them from destruction. For years, scientists believed that the proteasome only degraded proteins tagged by a 'death marker' named polyubiquitin, which directed damaged proteins to a complex that opened the gate. It was thought that substrates accessed the internal catalytic sites of the proteasome by processively threading their termini through the gated substrate channel. However, new findings reveal that some important substrates do not need to be marked with polyubiquitin, but can open the gate themselves, enter the active cylinder and be degraded.

The scientists conducted the research by performing biochemical assays using a purified protein involved in Parkinson's disease and a cell-cycle regulator important for the progression of cancer. They found that the proteasome could independently degrade these proteins, cutting in the middle of substrates at internal peptide bonds in an endoproteolytic process. These findings may have implications for the development of future drugs to treat these diseases.

1359-6446/03/$ – see front matter PII: S1359-6446(03)02617-5

2 Liu, C-w. et al. (2002) Endoproteolytic activity of the proteasome. Science 10.1126/science.1079293

Nicotine – a bigger deal than we thought?

Tobacco related diseases are a big killer with 4.2 million deaths per year attributed to the effects of tobacco smoke. Until now, scientists have only studied the genotoxic effects of the active ingredients in tobacco, but a recent study looking at the effects of two constituents of tobacco smoke on cellular pathways suggests that the effects could be worse that previously thought.

Philip Dennis and colleagues at the National Cancer Institute (http://www.cancer.gov) have published a study [3] that describes the effects of tobacco on signalling pathways in normal cells. They show that two components of cigarette smoke, nicotine and the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), activate the Akt pathway in non-immortalized human airway epithelial cells *in vitro* and that this could promote cancer development and progression.

The study shows that the stimulation of lung epithelial cells with amounts of nicotine and NKK equivalent to those seen in smokers, resulted in the activation of the Akt pathway promoting cell growth and survival in healthy cells. They also reported that the Akt pathway was active in the lungs of mice treated with NKK and in lung cancer tissue from smokers. This finding is significant, as the body's main defence mechanism against cancer is a cell's ability to maintain its 'normal' status and to die if irregularities occur. If the cell continues to grow in an unregulated way, mutations in genes such as *p53*, *p16*, or *K-ras* can occur and culminate in the formation of a tumour.

The identification of pathways that promote cellular survival before causing DNA damage highlights crucial steps in the process of carcinogenesis and further implicates nicotine and its derivatives in the causation of cancers. It might therefore be necessary to re-evaluate the risks versus benefits of nicotine therapy quitting aids, such as nicotine patches and chewing gum.

3 Kip, A. *et al.* (2003) Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J. Clin. Invest.* 111, 81–90

Miscellaneous

New insights into Viagra deaths

An estimated 16 million men worldwide have used Viagra®. A few hundred of them have died from heart attacks or strokes that have been attributed to the drug. This has baffled scientists because previous studies have indicated that Viagra® should prevent aggregation of platelets, which is known to underlie these conditions. Now, researchers led by Xiaoping Du of the University of Illinois at Chicago (http://www.uic.edu/) have shed new light on the mechanism of platelet clumping, helping to explain the deaths and possibly reduce the risk of fatality [4].

Aggregation of platelets is crucial for clotting at wounds, but inappropriate clumping of platelets can block blood vessels and lead to heart attack or stroke. For the past 20 years, it has been thought that cGMP and the enzyme it activates, cGMP-dependent protein kinase (PKG), inhibit platelet aggregation. However, Du and colleagues found that PKG could activate proteins that enhance aggregation *in vitro* and that platelet responses were impaired in PKG-deficient mice. Du and colleagues reconcile their results with previous findings by suggesting that cGMP-induced platelet responses are biphasic, initially causing aggregation (to plug a wound) but subsequently inhibiting it (so that blood flow is unhindered).

Because sildenafil (Viagra®) increases levels of cGMP, Du and colleagues went on to test whether this also promotes platelet aggregation. The drug was not sufficient to cause platelet aggregation by itself but it did enhance clumping in the presence of clotting factors at concentrations normally too low to cause aggregation but that can be present if blood vessels are damaged. This helps to explain how death could result. 'Viagra, by itself, probably is not sufficient to cause a heart attack in healthy people', explained Du, 'but our research suggests that it may present a risk for patients with pre-existing conditions such as atherosclerosis.'

4 Li, Z. *et al.* (2003) A stimulatory role for cGMP-dependent protein kinase in platelet activation. *Cell* 112, 77–86

Kinome map progress

First there was the Human Genome Project. Then came the Human Kinome Project. An ambitious collaboration between two organizations claims to have catalogued the whole complement of kinase genes in the human genome [5]. The team thinks their work will usher in a new area of drug research that could revolutionize cancer treatment and provide an alternative to chemotherapy.

Scientists from the Salk Institute for Biological Studies (http://www.salk.edu/) teamed up with the San Francisco based biotechnology company SUGEN (http://www.sugen.com/) to take on the challenge of studying protein kinases. When defective, kinases can lead to cancer progression.

Team member Sucha Sudarsanam said that previous attempts to map kinases were incomplete and described the publication of the human genome as 'the real breakthrough' that enabled them to make their findings.

The group has reportedly identified 518 putative protein kinase genes, of which 71 had not been previously published or described as kinases. They are also believe that kinases make up approximately 1.7% of human genes.

The next step towards treating cancer, says collaborator Tony Hunter, is 'to use this information for diagnostic and therapeutic purposes. Kinase inhibitors,' he hopes, 'will be major players in the next generation of targeted drugs for cancer and other diseases.'

5 Manning, G. *et al.* (2002) The protein kinase complement of the human genome. *Science* 298, 1912–1934

Gene therapy

Successful siRNA delivery

Scientists from the University of California at Los Angeles (http://www.ucla.com) and Caltech (http://www.caltech.com) have developed a novel gene therapy technique that delivers small interfering RNA (siRNA) into cells to prevent HIV from entering [10]. The therapeutic potential of siRNA to reduce the expression of specific genes and slow the progression of disease has long been known, but until recently there was no effective method for introducing the siRNAs into cells in a stable form.

Irvine S.Y. Chen, Director of the UCLA AIDS Institute (http://www.medsch.ucla.edu/aidsinst), and David Baltimore, Nobel Laureate and President and Professor of Biology at Caltech, collaborated to overcome this problem by creating a delivery vehicle derived from a disarmed version of HIV itself – a lentivirus vector, which is known to infect non-dividing cells in a stable manner.

The vector was targeted to mRNA for the HIV coreceptor CCR5 – individuals that lack CCR5 (approximately 1% of Caucasians) are resistant to HIV-1 infection, but are otherwise phenotypically normal. Human T cells extracted from healthy human blood were first transduced with the lentiviral vector containing siRNA targeted to CCR5 and then incubated with HIV-1 for eight days. Analysis of the cultures showed a 10-times reduction in CCR5 expression, and HIV infection was observed in less than 20% of cells.

These results have wide ranging implications. 'Our findings raise the hope that we can use this approach or combine it with drugs to treat HIV in humans – particularly in people who have not experienced good results with other forms of treatment', noted Baltimore. However, now that the stable transfection of siRNA has been demonstrated, this technology could become a major therapeutic approach for the treatment of many diseases in the future: 'This technology can be used to treat tumours or any disease in which a scientist wishes to knock out a malfunctioning gene' said Chen.

10 Qin, X.-F. *et al.* (2002) Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNS against CCR5. *Proc. Natl. Acad. Sci. U. S. A.* 100,183–188

Incorrect splicing: getting to the ESSENCE of the problem

When improperly spliced messenger RNA is translated, the resulting proteins can be defective, causing disease. A technique recently developed at Cold Spring Harbor Laboratory (http://www.cshl.org/) has the potential to remedy such splicing errors [11], possibly leading to therapies for diverse diseases of genetic origin.

Newly transcribed RNA is a 'word-for-word' copy of DNA and must be spliced to remove unwanted segments. In many genetic disorders, mutations can cause errors in splicing and the resulting proteins are ineffective. To address the problem, Adrian Krainer and Luca Cartegni of CSHL manipulated the cell's splicing machinery by tailoring SR proteins, which are enzymes used to correctly reassemble mRNA. SR proteins comprise a binding domain that attaches to the pre-mRNA transcript, and a domain that recruits enzymes to perform the cutting and pasting.

Krainer and Cartegni attached the recruiting portion to a specially designed segment that specifically binds to a target pre-mRNA by Watson–Crick base-pairing. Important parts of genes that are normally skipped in mutants can therefore be restored. Krainer was able to restore correct splicing of a mutant form of the *BRCA1* gene. Thus, the designed molecules corrected the splicing error, making normal mRNA from a defective transcript. Similar success was found when the technique was turned to the *SMN2* gene, which is associated with a neurodegenerative disease.

The technique, dubbed 'ESSENCE' for exon-specific splicing enhancement by small chimeric effectors, has only been demonstrated *in vivo* but shows much promise in a wide range of diseases. 'There are a lot of hurdles to overcome in terms of delivering the corrective molecules to the cells that need to be treated,' according to Brenton Gravel, a molecular biologist at the University of Connecticut Health Center (http://www.uchc.edu), not connected with the study, 'But theoretically the exact same approach could be taken for any gene at all.'

11 Cartegni, L. and Krainer, A.R. (2003) Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nat. Struct. Biol.* 10.1038/nsb887 (http://www.nature.com)

RNA, but not as we know it

Creation of a 'binary' RNA enzyme (ribozyme) has provided insight into the origins of life. John Reader and Gerald Joyce of the Scripps Research Institute (http://www.scripps.edu/) have generated a functional ribozyme that consists of just two different nucleotides, rather than the usual four [6]. Their work demonstrates that life could have existed with fewer building blocks than previously thought and helps to explain how the four-base nucleotide code might have originally developed.

It is not clear how the complexity of the chemical reactions that occur within cells could have evolved. RNA molecules are thought to have been important early on in evolution, as they can both encode genetic information and have catalytic activity. Furthermore, they are generated using combinations of just four building blocks. However, one of the four nucleotides, cysteine, degrades readily and might not have been stable enough for RNA to have started out with the four-base system.

Joyce has previously shown that the R3 ligase ribozyme could be fully functional, despite containing only three of the four RNA building blocks (adenine, guanine and uracil). Now, he and Reader have created a ribozyme made up of only two different bases, uracil and diaminopuridine (modified adenine). The string of nucleotides folded into a 3D structure that could catalyze a biologically relevant reaction, forming

3′5′-phosphodiester linkages between two RNA molecules at a rate 36,000 times that of the uncatalyzed reaction.

This study gives us a big clue as to what could have existed before the evolution of RNA as we know it today. 'Nobody will ever top this because binary systems are the most reduced form of information processing', says Joyce.

6 Reader, J.S. and Joyce, G.F. (2002) A ribozyme composed of only two different nucleotides. *Nature* 420, 841–844

Are drug companies developing new diseases?

An article published recently in the *British Medical Journal* [BMJ; 7] reports that during the past six years, researchers, sponsored mainly by the pharmaceutical industry, have been developing and defining new diseases characterized by female sexual dysfunction.

One of the milestones in the process where 'difficulties' become 'dysfunctions' that can then be termed 'diseases' in need of pharmaceutical intervention, was a study published in the *Journal of the American Medical Association* (JAMA) [8]. This study produced the now widely quoted figure that 43% of women aged between 18 and 59 experience sexual difficulties. This identified a large number of women who are currently not receiving any 'treatment' for these difficulties and thus a huge opportunity for drug companies to develop drugs for this market and increase profit growth.

Even though the JAMA article included the caveat that its data was 'not equivalent to clinical diagnosis' this is often overlooked and many researchers are worried about the communication of these results and its effects on the drug market. The corporate sponsored definitions of sexual dysfunction are being criticised by some as inaccurate and potentially dangerous.

The article in the BMJ reports that there are potential risks in the development of the term 'disease' when it is sponsored so heavily by pharmaceutical companies. The emphasis is on increasing profit and following the success of Viagra which, since its launch, has been prescribed to more than 17 million men to treat erectile dysfunction. If alternative treatments can be developed to treat female sexual dysfunctions that match Viagra sales (Pfizer reported profit of over £1.6 bn in 2001) the concern is that it might cause people to overlook the complex social, personal and physical causes of sexual difficulties in the rush to diagnose and prescribe.

However, there are some potential benefits in the medicalization of female sexual dysfunction because of its potential to generate a more holistic doctor–patient relationship, the development of safe and effective treatments for conditions that are often taboo, and an increase in public awareness and research attention to the complexity of female sexual problems.

Many researchers are reported to be concerned by the overmedicalization of female sexual dysfunction. Sandra Leiblum, Professor of Psychiatry at Robert Wood Johnson Medical School (http://rwjms.umdnj.edu) spoke at a recent New York educational workshop and said, '…there is dissatisfaction and perhaps disinterest among a lot of women, but that doesn't mean they have a disease'.

7 Moynihan, R. (2003) The making of a disease: female sexual dysfunction. *Br. Med. J.* 326,45–47

8 Laumann, E. *et al.* (1999) Sexual dysfunction in the United States: prevalence and predictors. *J. Am. Med. Assoc.* 281, 537–544 (published erratum appears in *JAMA* 281, 1174)

The mitosis 'on' switch: buzzer or toggle?

Researchers from Virginia Polytechnic Institute (http://www.vt.edu) have successfully validated the predictions of a mathematical model of cell cycle progression [9]. The progression of a cell through mitosis occurs as an irreversible transition — if the cell slips backwards into interphase before the chromosomes are properly separated, cancer-causing mutations may occur. In *Xenopus laevis*, the decision to enter or leave mitosis is mediated by the activation of the cyclin-dependent kinase Cdc2, which is activated by the accumulation of cyclin B. Cdc2 is inactivated when cyclin B is degraded, resulting in exit from mitosis.

The precise mechanism of this 'on/off' switch has yet to be established; in theory it could function either as a 'buzzer' or a 'toggle'. John Tyson, an author on the paper explains: 'A buzzer is on when one pushes hard enough on its button and it stops buzzing when one lets go. A toggle likewise switches on when one pushes hard enough on the lever in one direction, but it will stay on when one lets go. To switch a toggle off, one must push the lever with sufficient force in the opposite direction'. The toggle mechanism is known as hysteresis, and the model has been tested experimentally by Jill Sible and co-workers according to three defined parameters: the amount of cyclin required to start, maintain and stop mitosis.

Using cell-free egg extracts from frog, the researchers manipulated the concentration of cyclin necessary for entry into and exit from mitosis. The concentration necessary to trigger entry into mitosis was measured at 40 units of cyclin. Once mitosis was switched on, cyclin concentration could drop to 20 or 30 units, but Cdc2 remained activated and mitosis persisted. Cyclin concentration had to drop to 16 units or less before the mitosis switch was turned off, indicating that the control system is bi-stable and hysteretic.

The investigation yielded further results: 'If we raised the cyclin level modestly (beyond what was needed to trigger mitosis), we eliminated the checkpoint that normally halts the cell cycle if there is something wrong with the DNA' stated Sible.

The similarity of cell cycle components in frog and human cells means that the researchers now hope to develop and validate a model of human cell-cycle progression, which would not only further our understanding of cell-cycle regulation but also suggest new ways to control cell cycle disorders such as cancer.

9 Sha, W. *et al.* (2002) Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc. Natl. Acad. Sci. USA* 10.1073/pnas.0235349100 (epub ahead of print; http://www.pnas.org)

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