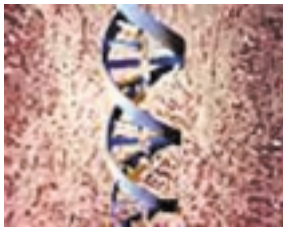
News in brief

Targets and mechanisms

DNA all locked up



Research chemists have discovered a class of synthetic molecules that could lock away portions of DNA, therefore preventing proteins from interacting with DNA in ways that could be detrimental biologically [1]. This can enable scientists to stop particular sequences of DNA from activating biological changes that need to be regulated closely or avoided.

Previously, researchers have only been able to produce small molecules that bind to the smaller, minor groove of DNA and can only stretch across a couple of base pairs. However, researchers in the Department of Chemistry at the University of Warwick (Coventry, UK) have recently produced a large synthetic molecule, or supramolecular cylinder, which can bind to the major groove of DNA. Upon binding, the molecule bends the section of DNA it is attached to, so the DNA becomes tightly coiled together, resembling the way in which non-synthetic molecules package DNA on the chromosome.

The team of chemists, led by Mike Hannon and Allison Rodger, designed the tetracationic metallo-supramolecular cylinder $[Fe_2L_3]^{4+}$, which is an iron triple helicate with three organic strands wrapped around two iron centres, and which targets DNA with a binding constant in excess of 10^7 M^{-1}. The two enantiomers of the molecule bind differently to DNA and induce different effects: the M enantiomer induces dramatic intramolecular coiling, whereas binding is less dramatic with the P enantiomer.

The strong binding mechanism to the major groove could also be used to enhance treatments using drugs that act on DNA where such drugs must not only be delivered into the correct cell but also into the nucleus. The current range of the molecule is 5 base pairs and the researchers are working to increase this to 15 base pairs and also to increase the specificity of the molecule.

1 Meistermann, I. *et al.* (2002) Supramolecular chemistry and self-assembly special feature: Intramolecular DNA coiling mediated by metallo-supremolecular cylinders: differential binding of P and M helical enantiomers. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5069–5074

Dynamic combinatorial chemistry

Novel neuraminidase inhibitors have been generated using dynamic combinatorial chemistry and could represent the first time that new enzyme inhibitors have been created using vitrual combinatorial libraries under the 'selection pressure' of a biological ligand [2]. Dynamic combinatorial chemistry is an emerging technique where equilibrated virtual combinatorial libraries are generated by combining building blocks capabale of undergoing reversible reactions. In the presence of a target ligand, the dynamic equilibrium is shifted towards the generation and amplification of the best-binding components of the library [3].

Researchers from Therascope (Heidelberg, Germany) added the target, namely the active site-containing extracellular domain of influenza A virus neuroaminidase, to a library composed of a family of aldehydes bound to a diamine scaffold. Addition of the influenza target shifted the equilibrium of the mixture in favour of the best-binding components, amplifying these neuroaminidase inhibitors by at least 120 times.

2 Hochgurtel, M. *et al*. (2002) Target-induced formation of neuraminidase inhibitors from *in vitro* virtual combinatorial libraries. *Proc. Natl. Acad. Sci. U. S. A.* 99, 3382–3387

3 Otto, S. *et al.* (2002) Dynamic combinatorial chemistry. *Drug Discov. Today* 7, 117–125

Protein chit-chat

Physicists have discovered how proteins interact to be able to perform the precise functions that occur at the cellular level [4]. The scientists found that protein–protein interactions are not random but are highly organized, at least in yeast cells.

Sergei Maslov, a physicist at the US Department of Energy's Brookhaven National Laboratory (Upton, NY, USA), said: 'Although scientists understand how a given protein interacts with other proteins, the way they connect with each other as a whole remains mysterious.' Maslov, together with Kim Sneppen of the Norwegian University of Science and Technology (Trondheim, Norway), used computer modelling to elucidate the interactions between proteins. They collected data on the protein–protein interactions among 6000 yeast proteins that exist on the public database and compared the resulting interaction network with a computer-simulated pattern of random protein interactions. They found that proteins that are highly connected to others are unlikely to 'talk' directly to other highly connected proteins; in fact, this type of interaction is systematically suppressed. By contrast, interactions between a highly connected protein and a low-connected protein are favoured.

Maslov described this phenomenon using the analogy of airline networks: 'Each airline company has a network of flights connecting different cities, but when a city serves as a hub for one company, the neighbouring cities are mostly served by this company,' he explains. 'Also the hub is served mainly by this company and not by another big company. So the two companies rarely ''talk'' to each other.' Maslov believes that hubs of interacting proteins are not likely to exist by chance, and the fact that they have been observed in yeast cells reveals a property of the cells that enables them to coordinate protein function. Studying these protein interactions can identify these coordinated functions, as well as intrinsic features of the proteins involved.

4 Maslov, S. and Sneppen, K. (2002) Specificity and stability in topology of protein networks. *Science* 296, 910–913

DNA: order at the back!

Researchers at Cornell University (Ithaca, NY, USA) have discovered how DNA moves in and out of small spaces, which could lead to a method for its separation by length and could show how large biological molecules move when confined [5]. The scientists used a forest of nanofabricated pillars that were so small that the DNA could only move sideways

1359-6446/02/$ – see front matter PII: S1359-6446(02)02340-1

between them. This revealed an entropic recoil force that causes the molecules to move from one confined space to another.

In previous studies, the team found that when a DNA molecule in a spherical configuration encounters an opening that is too small for it to move through, a small part is pulled into the opening and the rest uncoils and follows. The application of an electronic field causes the DNA to behave in the same way, but the question was, 'What causes this behaviour?' An entropic force – the amount of disorder in a system – is thought to be responsible for this movement as it tends to move things towards the most disordered arrangement.

The experiments involved the use of electron beam lithography at the Cornell Nanofabrication Facility, to build a silicon nitride device consisting of an array of pillars adjacent to an open space. The pillars were 35 nm diameter and 125 nm apart and the movement of the DNA was measured by fluorescence microscopy. Analysis of videomicrographs showed that the recoil was not elastic and, as Turner explains, happens because atoms within the DNA chain are in continuous motion, colliding both with water and the pillars. Between the pillars, these collisions occur in all directions and cancel each other out, but at the interface between the pillars and the open space, these collisions can only happen in one direction, which causes a force to be exerted that pulls the chain back out of the pillared space.

Turner said, 'What we have seen here is a new way in which disorder can force something to move.' The scientists say that these conclusions should apply to all long-chain molecules or polymers and suggest the entropic forces could have a role in the movement of molecules in living cells. It could also be used to separate molecules by length because any molecules that have moved into refinement do not subsequently recoil.

5 Turner, S.W. *et al.* (2002) Confinement-induced entropic recoil of single DNA molecules in a nanofluidic structure. *Phys. Rev. Lett.* 88, 128103

Gut bacteria are good for you

Administration of a synthetic form of bacterial DNA – immunostimulatory oligonucleotide (ISS-ODN) – to mouse models of inflammatory bowel disease (IBD) leads to amelioration of the disorder, with concomitant stimulation of the

Leptin and obesity

Leptin signalling in obesity

Protein tyrosine phosphate 1B (PTP1B) could negatively regulate leptin signalling and so provide a mechanism by which obesity can be regulated [10,11]. Although it is well established that a mutation in leptin or the leptin receptor causes obesity, leptin itself has not proved useful as a treatment for obesity. In fact, in many obese indiviuals, there are increased blood levels of leptin, suggesting resistance to the protein. However, two recent studies have identified a novel protein that regulates leptin signalling, which could provide a promising new target for the treatment of leptin resistance in obesity.

Researchers from McGill University (Quebec, Canada) found that leptin-deficient ($Lep^{ob/ob}$) mice lacking PTP1B exhibit an attenuated weight gain, a decrease in adipose tissue, and an increase in resting metabolic rate compared with mice lacking only leptin [1]. They were also shown to have an enhanced response toward leptin-mediated weight loss and suppression of feeding.

Meanwhile, researchers from Beth Israel Deaconess Medical Center and Harvard Medical School (Boston, MA, USA) showed that PTP1B is present in the leptin-responsive cells of the hypothalamas and that PTP1B deficiency reduced the weight gain seen in mice whose leptin signalling was disrupted [2]. The results from both these studies suggest that inhibitors of PTP1B could provide a useful alternative or supplement to leptin in the treatment of obesity due to leptin resistance.

10 Cheng, A. *et al.* (2002) Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev. Cell* 2, 497–503

11 Zabolotny, J.M. *et al.* (2002) PTP1B regulates leptin signal transduction *in vivo*. *Dev. Cell.* 2, 489–495

Protein identified that overcomes leptin resistance

Scientists have moved a step closer to developing a drug to prevent and treat obesity, with the identification of a protein that helps the body to overcome resistance to leptin [12].

Obesity is becoming a major health problem in the developed world, and most obese individuals are resistant to the hormone leptin, which usually signals to the brain when appetite is satiated. Although leptin is often present in high levels in obese people, they are unable to use it.

The scientists, led by Barbara Kahn and Benjamin Neel, both of Harvard Medical School (Boston, MA, USA), created knockout mice that lacked protein tyrosine phosphatase 1B (PTP1B), which is thought to have a role in insulin receptor signalling. They hypothesized that, without PTP1B, the mice would experience increased insulin sensitivity and protection against type 2 diabetes mellitus.

The group found that not only did the mice become hypersensitive to insulin, but that they were also surprisingly lean. When fed a high-fat diet, the mice gained less weight and had markedly reduced body fat compared with a control group. 'They turned out to be resistant to a high-fat diet that is equivalent to what most Americans probably routinely consume', said Neel. The mice also showed increased energy expenditure, 'comparable to highly trained athletes', he commented.

The researchers suggest that PTP1B, while also playing a role in insulin receptor signalling, also regulates the leptin signalling pathway. Indeed, they have shown in transfection studies that PTP1B dephosphorylates the leptin receptor-associated kinase, Jak2 [13]. Because most forms of obesity show leptin resistance, it might be possible to develop a drug based on PTP1B that acts downstream of the leptin receptor, and which could lead to significant weight loss. Neel adds that, 'since PTP1B additionally acts to lower insulin resistance, it could, in principle, provide a very powerful approach to treating diabetes as well.'

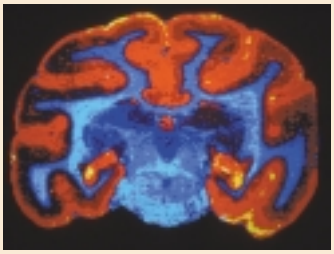
On a similar topic, researchers from Myriad Genetics (Salt Lake City, UT, USA). have recently published the location of a predisposition gene for obesity, located at 4p15–p14 [2].

12 Zabolotny, J.M. *et al.* (2002) PTP1B regulates leptin signal transduction *in vivo*. *Dev. Cell.* 2, 489–495

13 Stone, S. *et al.* (2002) A major predisposition locus for severe obesity, at 4p15-p14. *Am. J. Human Genet.* 70, 1459–1468

CNS-related disorders

A possible halt to Huntington's disease



A protein developed by researchers from University of California, Irvine (CA, USA) and Massachusetts Institute of technology (MIT; MA, USA) has been shown to halt the progression of Huntington's disease in a *Drosophila* model, and could help scientists to find ways of using gene therapy to halt or even prevent the disease [14].

Huntington's disease is a genetic neurogenerative disorder characterized by cognitive defects, psychiatric symptoms and movement problems. It is caused by an expansion of a polyglutamine repeat in the huntingtin protein (Htt) that results in neuron degeneration in the CNS, and visible protein aggregates within neurons.

The researchers created suppressor polypeptides that, when tested in cell cultures, bound to the mutant Htt, reducing levels of the lethal protein and thus interfering with the aggregation process. When tested in *Drosophila*, the most potent suppressor inhibited the degeneration of photoreceptor neurons, possibly by preventing the protein from aggregating, suggesting that the suppressor can disrupt the molecular interactions that eventually lead to Huntington's disease.

To combat the disease in humans, the Htt needs to be blocked in all the nerve cells that are affected by the disease. Gene therapy is one method that could be used to do this, although there are still several obstacles to using such technology, particularly for suitable delivery of the beneficial gene into the relevant cells. However, gene therapy is the subject of intensive research and, as Larry Marsh, one of the authors of the paper and Professor of Developmental Biology in the School of Biological Sciences at MIT hopes, the suppressor 'may be readily administered using more traditional medical techniques'.

14 Kazantsev, A. *et al.* (2002) A bivalent Huntingtin binding peptide suppresses polyglutamine aggregation and pathogenesis in *Drosophila*. *Nat. Genet.* 30, 367–376

Brain cancer vaccine shows promising results

An experimental vaccine for brain cancer has shown encouraging results in a preliminary study at University of California at Los Angeles Jonssen Cancer Center (CA, USA) [15]. The vaccine completely prevented brain tumor formation in laboratory rats that had received the vaccine, whereas rats that did not receive it developed aggressive tumours.

There is an urgent need for effective treatments for brain cancer, which is almost 100% fatal. 'Without any treatment, patients with the most aggressive gliomas usually do not live longer than nine months. Even after surgery, radiation and chemotherapy, patients usually live only for as long as two years,' said Liau.

Researchers used lymphocytic choriomeningitis virus nucleoprotein (LCMV-NP) as a 'pseudotumour antigen', an antigen known to be recognized by the immune system, and partnered it with an attenuated form of the bacterium *Listeria monocytogenes*. The bacteria served as a transportation mechanism for the antigens.

'The immune system is already primed to fight bacteria. So by using specially engineered bacteria to transport the antigens, we drew the immune system's attention to the tumour antigens,' Liau said, 'and with its attention focused on the antigens, the immune system learned to recognize and attack the cancer cells that produced those antigens.'

Although it will be several years before human testing can begin, Liau is optimistic that targeting the immune system could be the key to improving treatment of brain cancer: 'I suspect that the body's immune system is more intelligent than anything we could configure to recognize foreign cells or agents, and more effective than traditional treatments at leaving healthy cells alone.'

15 Liau, L.M. *et al.* (2002) Tumor immunity within the central nervous system stimulated by recombinant *Listeria monocytogenes* vaccination. *Cancer Res.* 62, 2287–2293

immune system and no risk of infection [6]. Researchers at the University of California at San Diego (CA, USA), Shaare Zedek Medical Centre (Jerusalem, Israel) and Tel-Aviv Sourasky Medical Centre (Tel-Aviv, Israel) have shown that synthetic DNA prompts the immune system to safely respond to the onset of IBD, according to the study's senior author, Eyal Raz.

The cause of IBD is unknown but thought to be multifactorial and is related to the much more serious Crohn's disease and ulcerative colitis (UC). It affects approximately one-million people in the USA, but is rare in the developing world. In most cases, symptoms include abdominal discomfort and diarrhoea, but more serious cases can require surgery to remove part of the bowel.

The results of the study by Raz and coworkers, support the 'hygiene hypothesis': that allergies and other immune disorders are a consequence of our ultraclean western world of vaccines, antibacterials and antibiotics, where we are unnecessarily protected from harmless bugs. Our immune systems, out of practice, are prone to over-reaction on exposure to relatively harmless pathogens, attacking both pathogen and our own tissues, leading to the inflammation seen in IBD and other disorders.

ISS-ODN was tested orally and systemically in four mouse models of IBD: three that were either chemical or hapten-induced colitis, and one spontaneous form in interleukin-10 knockout mice. The symptoms of IBD – induction of pro-inflammatory cytokines and chemokines, and induction of colon matrix metalloproteases – were inhibited on administration of ISS-ODN to all mice models. However, the effect was only transient and further administration of the DNA was required to allow the mice to remain disease-free.

It is hoped that these results could be extended to human clinical trials, leading potentially to a once-weekly pill for IBD patients to prevent the sudden bouts of inflammation that typify the disease. The effects of ISS-ODN in Crohn's disease and UC might also yield interesting results.

6 Rachmilewitz, D. *et al.* (2002) Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology* 122, 1428–1441

Natural compound reduces cholesterol

A natural compound derived from the guggul tree (*Commiphora mukul*) helps to

reduce low-density lipoprotein (LDL) cholesterol, according to researchers at the University of Texas Southwestern Medical Center Medicine (Dallas, TX, USA) and Baylor Colege of Medicine (Houston, TX, USA) [7]. Guggulsterone (4,17[20]-pregnadiene-3,16-dione) is the active sterol of the guggul tree, the gum resin of which has been used in Ayurvedic medicine in India since 600 BC. An ethyl acetate extract of the resin (gugulipid) was approved in India in 1987 as a hypolipidaemic agent and has been used successfully to lower LDL cholesterol and triglycerides in humans.

The release of cholesterol from the liver in the form of bile acids is controlled by a negative feedback loop involving the farnesoid X receptor (FXR). FXR is a promiscuous nuclear hormone receptor, however, and can be activated by several other compounds not structurally related to bile acids.

Guggulsterone acts as a highly efficacious FXR antagonist *in vitro* and *in vivo*. This was first shown in transient transfection assays where guggulsterone directly blocked FXR activity. Guggulsterone treatment was then shown to reduce hepatic cholesterol in wildtype mice fed a high cholesterol diet but not in FXR-null mice.

This work could lead to new drugs to reduce LDL cholesterol by designing compounds based on guggulsterone. Several other natural compounds known to affect cholesterol metabolism through unknown mechanisms could also act via FXR and related receptors and might also be potential targets for drug development.

7 Urizar, N.L. *et al.* (2002) A natural product that lowers cholesterol as an antagonist ligand for the FXR. *Science* 10.1126/science.1072891

Cardiotoxicity and anti-cancer drugs

Culprit found for Herceptin-mediated heart failure

Researchers have identified the probable cause of cardiac failure resulting from the use of the breast cancer drug Herceptin [8]. Scientists at the Salk Institute (La Jolla, CA, USA) and researchers at the University of California at San Diego (UCSD; CA, USA), discovered the link that could explain why a common combination drug regimen, which includes Herceptin, is toxic.

Herceptin targets the protein Her2 (a human epidermal tyrosine kinase receptor), which is expressed in certain breast cancer cells to a high level. This study has shown that the mouse homologue, erbB2 (related to an oncoprotein in erythroblastosis virus B) is necessary for proper functioning of the heart.

Kuo-Fen Lee, a professor at the Salk Institute and senior author of the study, said: 'It was possible that Herceptin triggered cardiac malfunction by a number of mechanisms, but now we know it appears that the drug's direct action on erbB2 is the culprit.' He also added that it might be possible to engineer novel drugs that could decrease the effects caused by Herceptin (such as enlargement and impaired contraction of the heart).

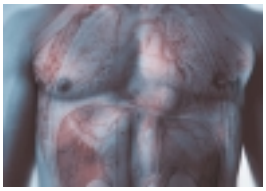
The group engineered mice to have a ventricular-restricted deletion of *Erbb2*. Previous work showed that knockout mice that were totally lacking in erbB2 would not survive because of developmental defects. Steve Crone, co-lead author of the study, said: 'To look at fully developed animals, which would most closely resemble patients, we set up the *erbB2* gene so that it would shut off after birth, and only in the heart.' Mouse hearts were examined at one and six months and cardiomyopathy was observed similar to that seen with Herceptin-related cardiac dysfunction.

Co-author Ken Chien, director of the Institute of Molecular Medicine at UCSD, said: 'The enlarged heart and impaired contraction of these *erbB2* mutant mice point to an unsuspected cardioprotective role for the secreted protein, neuregulin, which stimulates this receptor.' He continued, 'This mouse model will help us identify new mechanisms to protect patients from Herceptin cardiomyopathy, and thereby allow more aggressive and early use of Herceptin for a broad range of human cancers.'

Furthermore, the combination treatments for cancers often include anthracyclines. The team researched the effects of these drugs on the mutant mice and found that the cardiomyocytes were more sensitive to a certain anthracycline – adriamycin – at drug levels that mimicked the therapeutic dose used in humans. This suggests that it might be worth examining alternative drugs for use in combination with Herceptin.

8 Crone, S.A. *et al.* (2002) ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat. Med.* 8, 459–465

Lag time after chemotherapy could help prevent cardiotoxicity



Researchers at the University of Copenhagen (Denmark) [9] have found that assessing left ventricular function immediately after chemotherapy is unlikely to accurately predict cardiotoxicity induced by anthracyclines. Treatment with anthracyclines, such as epirubicin, results in heart failure in 10–15% of patients. Studies have shown that 65% of children treated for leukaemia develop cardiac abnormalities six years after completing treatment with anthracyclines.

In a long-term prospective study with epirubicin, the Danish research team, led by Benny Jensen, found a significant decrease in cardiac function three months or more after the start of chemotherapy and with nearly 60% of patients experiencing a 25% decrease in left ventricular function three years after treatment. After five years a fifth of the patients treated with high-dose epirubicin had developed severe cardiomyopathy. This lag time between the end of chemotherapy and deterioration in cardiac function suggests that monitoring should be continued for several months and even years after cessation of treatment. Furthermore, it could provide an opportunity for medical intervention to prevent heart failure occurring in later life. Jensen's group are now researching the hypothesis that angiotensin-converting enzyme (ACE) inhibitors can restore cardiac function in patients with breast cancer who had been treated with the drug epirubicin.

9 Jensen, B.V. *et al.* (2002) Functional monitoring of anthracycline cardiotoxicity: a prospective, long-term observational study of outcome in 120 patients. *Ann. Oncol.* 13, 699–709

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