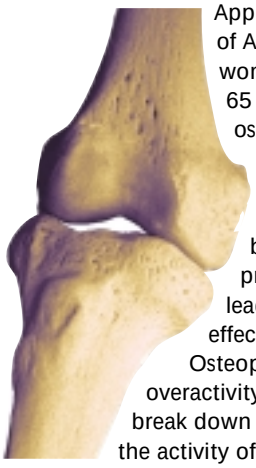
News in brief

Targets and mechanisms

New leads for osteoporosis research



Approximately ~50% of Asian and Caucasian women over the age of 65 suffer from osteoporosis. New insights into how the cells responsible for the breakdown of bone are generated [1] promise to provide new leads in the hunt for effective treatments. Osteoporosis reflects an overactivity of osteoclasts (which break down bone) relative to the activity of osteoblasts (which build bone). Scientists led by Steven L. Teitelbaum of the Washington University School of Medicine (http://medicine.wustl.edu/) had previously shown that macrophage-colony-stimulating factor (M-CSF) can push undifferentiated cells into becoming mature osteoclasts, and that a lack of M-CSF causes animals to have abnormally dense bone. It had also been demonstrated that blocking the function of $\alpha_v\beta_3$ integrin in animals inhibits osteoclast activity. Now, Teitelbaum and colleagues have begun to reveal the molecular mechanisms underlying these effects.

Consistent with the earlier findings, Faccio *et al*. found that absence of the β_3 part of $\alpha_v\beta_3$ integrin from bone precursor cells inhibited the development of functional osteoclasts *in vitro*. However, *in vivo* the β_3-deficient cells produced more (albeit dysfunctional) osteoclasts than normal. As Teitelbaum explains, 'This paradox suggests that something in the living animal interacts with β_3 during the process of osteoclast differentiation.' He and his colleagues have now shown that the extra factor is M-CSF. Adding M-CSF to *in vitro* cultures of β_3-deficient cells restored their ability to produce osteoclasts. The team went on to show that externally regulated kinases (ERKs) and c-Fos could mediate this effect of M-CSF, and they identified the part of the M-CSF receptor that is involved.

'The interaction between M-CSF and $\alpha_v\beta_3$ integrin is intriguing and may help explain some of the less-understood aspects of animal models of osteoporosis', says Teitelbaum. $\alpha_v\beta_3$ integrin is already a therapeutic target in the fight against osteoporosis but the work of Faccio *et al*. opens up new possibilities to those searching for ways to beat this debilitating disease.

1 Faccio, R. *et al.* (2003) C-Fms and the avb3 integrin collaborate during osteoclast differentiation. *J. Clin. Invest.* 111, 749–758

Tyrosine kinase involvement in pre-eclampsia

Pre-eclampsia is a serious complication affecting 5% of pregnancies, which causes substantial maternal and foetal morbidity and mortality. It is a major cause of premature birth and perinatal child death, also accounting for ~15% of all maternal deaths. Although the pathophysiology of pre-eclampsia remains largely unknown, two recent studies shed new light on the cause of this disease [2,3].

Blood flow to the placenta is reduced and the oxygen supply is decreased in pre-eclamptic women. Low oxygen levels have been proposed to trigger the release of unknown factors in the placenta that mediate a rapid and unpredictable progression to numerous multisystem complications, involving the maternal liver, kidneys, lungs, blood and nervous systems.

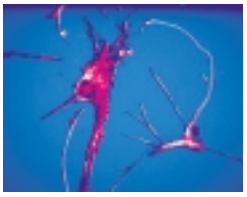
The first study, by researchers at the Beth Israel Deaconess Medical Center in Boston, MD, USA (http://www.bidmc.harvard.edu/), reports that elevated levels of circulating soluble fms-like tyrosine kinase 1 (sFlt1) contributes to the pathogenesis of pre-eclampsia [2]. Rats that had been treated with this protein developed several clinical and pathological features of pre-eclampsia, regardless of whether or not they were pregnant, thus providing evidence of a causal role for sFlt1.

sFLT1 is an antagonist of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and the second study reports that tight regulation of VEGF-A signalling is crucial for establishing and maintaining the glomerular filtration barrier and supports a role for VEGF-A in renal disease. The group from the Samuel Lunenfeld Research Institute in Toronto, Canada (http://www.mshri.on.ca/), also report that reducing the levels of VEGF-A in a subset of murine kidney cells causes kidney disease similar to that seen in pre-eclamptic women [3].

Together, these results suggest a mechanism for what goes wrong during pre-eclampsia and highlight several molecular pathways and molecules that could be potential targets for future therapeutic intervention.

2 Maynard, S.E. *et al.* (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J. Clin. Invest.* 111, 649–658
3 Eremina, V. *et al.* (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J. Clin. Invest.* 111, 707–716

Making the right connections



For the first time, researchers have identified a molecule that mediates the connection of nerve cells in the complex setting of a live animal. Kang Shen and Cornelia Bargmann of the University of California, San Francisco (http://www.ucsf.edu/) have reported a 'matchmaker' protein that guides the correct association of neurons in *Caenorhabditis elegans* [4].

Learning about the interactions that mediate nerve connection could have important implications for drug discovery. Potential targets include chronic epilepsy and pain, in which synapses form incorrectly. Such knowledge could also help in the repair of peripheral nerve damage – a complex task because any given neuron must connect with the correct partner, from many options. In Bargman's words, 'Like people, neurons can make good or bad choices about who they associate with.'

Molecules that direct synapse formation have been sort for many years and several

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candidates have been suggested, but this is the first to be found in a living animal. The protein, known as SYG-1, is a member of the immunoglobulin family and came to light during studies of the egg-laying behaviour of *C. elegans*. When synapses form, vesicles containing neurotransmitters congregate in the pre-synaptic neuron. By studying mutant mice, the researchers found that the vesicles clustered in the normal location even when the target neuron was missing. This contradicts the assumption that nerve connection is triggered by physical contact.

Looking more closely, they found that SYG-1 accumulates in the pre-synaptic neuron just before the vesicles form. They hypothesized that SYG-1 is a receptor for a signal from the egg-laying tissue of the worm. During development, SYG-1 acts as a 'guidepost', directing the two neurons to connect in the tissue close to the egg-laying muscles. A closely related protein has been detected in mice during brain development, and Bargmann suspects it will be found in humans too.

4 Shen, K. and Bargmann, C.I. (2003) The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. *Cell* 112, 619–630

Miscellaneous

Dendrons make it big

A liquid-crystal lattice that self-assembles from thousands of synthetic molecules holds promise as a drug-delivery vehicle. Scientists from the University of Pennsylvania (http://www.upenn.edu/) and the University of Sheffield (http://www.shef.ac.uk/) have reported the first organic compound to self-arrange into a structure whose intricacy rivals those of complicated metal alloys [5].

Dendrons are branched organic molecules that can self-assemble into large aggregates, known as dendrimers, under the right conditions. A team headed by Virgil Percec, from the University of Pennsylvania, investigated how the self-assembly process is dictated by the molecular structure of the dendrons. They started by combining carefully designed dendron building blocks, which were highly branched and tapered at one end. These self-assembled into spheres containing 12 dendrons, joined at the narrow ends.

The researchers found that 30 of these globular structures arrange into a tetragonal lattice, the repeating unit of which is a rectangular prism of over a quarter of a million atoms. This represents one of the most complex supramolecular structures ever formed by self-assembly. The dimensions of this prism are comparable to those of small virus particles. 'The achievement of a lattice of this size is a significant step towards designing new synthetic molecules which would form even larger structures, with dimensions approaching the wavelength of light,' commented Percec. Achieving this goal could yield a new type of photonic crystal, with applications in the telecommunications and electronics industries. The team is also experimenting with their dendron structure with the aim of producing a building block that self-assembles into hollow spheres. This would enable encapsulation of materials and could have applications in drug delivery and gene therapy.

5 Ungar, G. *et al.* (2003) Giant supramolecular liquid crystal lattice. *Science* 299, 1208–1211

Taking a better look at glaucoma

New findings have been reported on the genetic basis of inherited glaucoma. In mouse studies, researchers have discovered a further gene mutation that can exacerbate the effects of mutations previously linked to glaucoma [6].

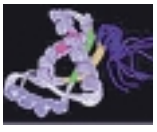
Glaucoma is a disease of the eye in which the ocular drainage structures that control pressure are malformed. It was already known that defects in the gene encoding a protein known as Cyp1b1 could cause glaucoma. However, unusual inheritance patterns had caused the researchers, led by Simon W. M. John, of the Howard Hughes Medical Institute (http://www.hhmi.org/), to suspect that multiple genetic and environmental factors were at play. For example, some infants who inherited primary congenital glaucoma (PCG), a particularly severe, although rare, form of the disease, showed acute effects from early life, whereas others showed delayed symptoms or none at all.

The researchers noticed that albino mice with the Cyp1b1 mutation were more affected than pigmented mice. Working on this clue, they found that the severity of disease in Cyp1b1-negative mice depended on the status of another gene, which codes for tyrosinase. This enzyme is a key catalyst in the pigmentation process, where it converts tyrosine to L-DOPA. Promisingly, administering L-DOPA alleviated the symptoms in mice. Other genes were then scrutinized for connections. Mice lacking the *FOXC1* gene, which has also been implicated in PCG, showed more severe abnormalities in the ocular drainage system than mice with the gene. In addition, it is thought that mutations in other genes in the L-DOPA pathway could affect the severity of glaucoma. As John noted, 'Our work provides a conceptual linkage for anterior segment developmental disorders caused by different genes.'

Although L-DOPA is already used as a drug to treat Parkinson's disease, the researchers are cautious about recommending its use for glaucoma treatment. John noted, 'L-DOPA is a molecule that affects the nervous system, and we need to proceed very carefully with animal and human studies before we will know whether such a treatment can become a clinical reality'. He added that drugs which target tyrosinase might be better therapeutics.

6 Libby, R.T. *et al.* (2003) Modification of ocular defects in mouse developmental glaucoma Models by tyrosinase. *Science* 299, 1578–1581

A shot across the bow for CJD



A promising line of research is under way that could yield preventative therapies against prion diseases, such as CJD. A team led by Simon Hawke of Imperial College London (http://www.ic.ac.uk/) have developed a monoclonal antibody (mAb) that appears to prevent the onset of prion disease in mice [7].

In the 17-month study, mice treated with mAbs remained healthy indefinitely, whereas their untreated counterparts succumbed to prion disease. Groups of mice infected with scrapie prions were administered doses of mAb that specifically

Cancer targets and mechanisms

How breast cancer fights back

Tamoxifen is widely used to prevent recurrence of breast cancer and to protect women at risk from the disease. However, the drug is not always effective. New results indicate that this is because the tumours of some women produce factors that block the ability of tamoxifen to fight cancer [9].

It is known that when AIB1 (amplified-in-breast-cancer 1; also known as SRC-3), a coactivator of oestrogen receptors, is overexpressed in cultured cells, the ability of oestrogen receptors to respond to tamoxifen is reduced. AIB1 can be phosphorylated and activated by signalling through the tyrosine-kinase receptor HER-2 (human epidermal-growth-factor-receptor 2; also known as neu or ErbB-2), and HER-2 is often overexpressed in human breast cancers. However, until now it has not been known whether increased levels of HER-2 and AIB1 can account for tamoxifen resistance *in vivo*.

C. Kent Osborne and colleagues at the Baylor College of Medicine (http://www.bcm.tmc.edu/) have now provided strong evidence that increased levels of HER-2 and AIB1 do, indeed, contribute to the resistance. They found that women with high levels of AIB1 and HER-2 had the worst responses to tamoxifen treatment. 'In fact, tamoxifen might have an adverse effect', said Osborne.

The team concluded that the efficacy of tamoxifen treatment in patients with breast cancer could depend, at least in part, on the levels of AIB1 and HER-2 in the tumour. Their findings suggest that AIB1 and HER-2 could be future targets for anti-breast-cancer treatments and that levels of these factors could be used to predict which women will respond positively to tamoxifen therapy.

9 Osborne, C.K. *et al.* (2003). Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J. Natl. Cancer Inst*. 95, 353–361

homed in on the prions. When the mAb treatment was given before neurological symptoms of scrapie appeared, the mice remained healthy. When the treatment was given after the onset of symptoms, however, no therapeutic effect was seen and the mice developed scrapie. It appears that, when administered early enough, the mAbs bind to the prion protein, preventing its conversion into the abnormal infectious form, thus halting the disease.

The researchers are keen to point out that the research is only at an early stage and any human trials are someway off. 'The work is a key scientific advance', said Hawke, 'but there is more development work to be done before we can begin to think about translating this research to the clinic'. Several issues have to be addressed first. So far, the work has only been demonstrated in mice. A humanized form of the antibody must be developed by genetic engineering to minimize the risk. In addition, because the mAbs are only therapeutic when introduced before the onset of disease, some way needs to be found of pre-symptomatically diagnosing diseases such as CJD; no such test currently exists. However, the researchers will be looking at whether this limitation can be negated by concentrating larger doses of mAbs in the brain, allowing treatment of the prion disease after symptoms have developed.

7 White, A.R. *et al.* (2003) Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature* 422, 80-82.

A new key to blocking inflammation

Scientists at the University of California, San Diego (http://www.ucsd.edu/) [8] have discovered that eliminating the ability of white blood cells to respond to low oxygen levels effectively blocks the development of inflammation in mice, an advance that could have widespread implications for the prevention of inflammation in humans.

At sites of injury the local vascular structure surrounding a wound is disrupted and the normal oxygen supply reduced, creating a low-oxygen (or hypoxic) environment. To enable white blood cells to function effectively under these conditions, they possess molecular and genetic 'switches' that allow them to change their metabolism from an oxygen-dependent (aerobic) to an oxygen-independent (anaerobic) mode of generating energy. By eliminating the ability of white blood cells — specifically macrophages and neutrophils — to turn on their hypoxic response and generate energy anaerobically, it should be possible to reduce inflammation.

The researchers produced three genetically modified strains of mice, each lacking a key factor in a biochemical pathway known to regulate the ability of cells to adapt to hypoxic environments. Testing the responses of the three different strains, they discovered that inactivation of a protein known as hypoxia inducible transcription factor-1α, or HIF-1α, strongly blocked the inflammatory response in mice. HIF-1α was shown to be essential for metabolism and energy generation of the white blood cells. In mice lacking a regulatory protein known as the von Hippel Lindau factor (VHL), which degrades HIF-1α in normal cells, the inflammatory response was heightened, presumably due to the large excess of HIF-1α that accumulated. It was also found that mice without HIF-1α could avoid the joint swelling and inflammation symptoms that typically occur when normal mice are subjected to an arthritis-inducing treatment. 'By altering the ability of these cells to operate normally in a low-oxygen environment, we've essentially prevented inflammation in mice,' noted Johnson, who headed the study. Their discovery could lead to the development of a new class of drugs for treating arthritis and may have wider implications for the treatment of cancer, as in many cases tumour development is associated with a pronounced inflammatory response.

8 Cramer, T. *et al*. (2003) HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* 112, 645–657

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