the C-terminal extension and used for substrate specificity studies. Protease inhibitors should be potent and selective for this enzyme to avoid side effects occurring through the inhibition of other physiologically relevant proteases.

Combinatorial chemistry libraries offer the potential to generate large numbers of compounds and screen for biological activity against the target of interest. Screening of these libraries and selecting the best hits can provide for new structural motifs to base further design strategies around. The development of one-bead-two-compound libraries allow the screening of millions of compounds in a competitive fashion in which each inhibitor in a single bead competes with a fluorogenic quenched substrate for binding to the protease. Because the synthetic strategy (split-and-mix) initially produce a unique structure on each bead to which a common fluorescence quenched substrate is attached, the library can be viewed as a collection of microreactors (with a volume of ~50 nL) that illuminates upon cleavage of the substrate when containing poor inhibitors. Potent inhibitors, in turn, can be identified by selecting the darkest beads where the substrate is intact due to high protease affinity for the inhibitor.

Recently the synthesis and screening of a combinatorial library of peptidotriazoles has been reported [2]. A library consisting of about half of 800,000 possible peptidotriazoles was synthesised on 450,000 beads. The library was incubated with *L. mexicana* CPB2.8ΔCTE. The fluorescence intensity was monitored and, after 24 hours, only a few beads remained dark. The long exposure to enzyme ensured only the most potent inhibitors were selected. After a sorting procedure, 48 hits (very dark beads) were selected. The inhibitors were re-synthesised in solution as C-terminal carboxamide with a free N terminus to mimic the inhibitors present in the solid phase library. Of these compounds, one of the most potent was (**vii**) with a Ki of 76 nM against against *L. mexicana* CPB2.8DCTE. This work has produced novel peptidotriazole compounds as promising inhibitors of *L. mexicana* cysteine protease and further work in this area is warranted.

H-Gly-RTr-ClF-Leu-Thr-Ile-Ser-Arg-Gly-NH_2

(vii)

2 Tornoe, C.W. *et al.* (2004) Combinatorial library of peptidotriazoles: identification of [1,2,3]-triazole inhibitors against a recombinant *L. mexicana* cysteine protease. *J. Comb. Chem.* 6, 312–324

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Biology

Neuroscience

Sour solution: postischemic neuroprotection via ASICs

Rising levels of intracellular calcium ions (Ca^{++}) in neurons after ischemia are believed to be responsible for the resulting brain damage. Up to now, NMDA receptors were thought to provide the main Ca^{++} entry pathway into the cell, and some clinical trails are currently underway or already completed, testing NMDA receptor antagonists for their neuroprotective effects after ischemia. Unfortunately, theses studies have shown rather disappointing results in protecting against brain damage after stroke in humans. Therefore, the question arises again: how do calcium ions enter the neurons after ischemia?

A recent paper published in *Cell* concentrated on this problem by considering the acidic environment that occurs during brain ischemia due to anaerobic glycolysis [1]. Increasing concentration of H^+ activates the acid-sensing ion channels (ASICs), which are known to be permeable to Na^+ and Ca^{++}. The subunits ASIC1a and ASIC2a are abundant in the brain and could provide a way of calcium ion entry that is independent of glutamate receptors. ASICs are sensitive to the sodium channel blocker Amiloride, whereas Psalmotoxin 1, a substance from venom of tarantula, has been shown to be a specific blocker of ASIC1a.

Xiong *et al.* exposed cultured mice cortical neurons to a pH of 6 and showed, by calcium imaging with fura-2, that the level of intracellular calcium is increased. The same effect occurs by depriving the cells of oxygen and glucose, which is an *in vitro* model for ischemia. Both methods led to increased cell death, which could effectively be inhibited by Amiloride and Psalmotoxin 1. Interestingly, neurons from ASIC1a-knockout mice displayed no increase in intracellular calcium ions when exposed to pH 6, and they seemed to be resistant against acid-induced cell death.

The authors induced a focal transient ischemia by occluding the middle cerebral artery in rats and mice. The evoked infarct volume was significant smaller in rats treated with Amiloride or Psalmotoxin 1. No treatment was necessary for the ASIC1-/- mice to show a strongly reduced infarct volume compared with wild-type mice. When combining Psalmotoxin 1 in wild-type mice with memantine, an uncompetitive NMDA receptor antagonist used in clinical trials, the authors could detect an additive effect. Thus, this study reveals the importance of the acid-sensing ion channel 1 in the development of brain injury after ischemia. These findings could help in the design of novel therapeutic neuroprotective strategies for brain ischemia.

1 Xiong, Z.G. *et al.* (2004) Neuroprotection in ischemia; blocking calcium-permeable Acid-sensing ion channels. *Cell* 118, 687–698

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Molecular Biology

It's a SNP

Variability within disease genes can influence the aetiology of resultant disorders and their amenability to treatment; with high-throughput genetic

screening, it is now possible to determine how specific polymorphisms within such genes impact upon the function of their protein products, and in turn, affect phenotype. Chen and colleagues have undertaken such a study by investigating the functional consequences of polymorphisms within a strong candidate gene for schizophrenia.

Schizophrenic subjects present with psychosis and abnormalities of affect and cognition. At the neurobiological level the disorder is characterized by abnormalities of dopamine metabolism in the prefrontal cortex. Success in determining the genetic basis of the disorder by linkage has been limited, but one region that has aroused interest is chromosome 22q11. 22q11 contains the COMT gene, whose product catalyses the transfer of a methyl group to a hydroxyl group on a catechol nucleus and thus could regulate dopamine metabolism. In the present study, the authors assayed the effects of four polymorphisms in the COMT gene on transcription/translation efficacy and enzymic activity using a large sample of schizophrenic brains; the four polymorphisms tested had previously been suggested to affect these parameters.

Although none of the polymorphisms examined significantly affected COMT mRNA levels, subjects homozygous for a G to A substitution [encoding a valine (Val) residue instead of a methionine (Met)] demonstrated greater COMT immunoreactivity than subjects homozygous for the 'Met' polymorphism. Moreover, COMT activity in the frontal cortex was significantly enhanced in Val/Val subjects relative to their Met/Met counterparts, a result that was apparently not simply due to raised protein levels in the Val/Val subjects, but rather to enhanced enzyme stability and performance (as indicated by *in vitro* assays of the two variant proteins). The effects elicited by the other three polymorphisms on protein levels and enzyme activity were significantly smaller, though there was an effect of polymorphisms in the promoter 2 sequence (encoding the membrane-bound COMT isoform) on enzymic activity.

Using elegant molecular methods the study provided additional compelling evidence for the functional importance of the Val/Met substitution on the activity of the COMT protein in the frontal cortex under physiological conditions. Clearly the next step must be to determine mechanism; the authors suggest that the surface Val residue, being relatively hydrophobic, might stabilize the enzyme structure more readily than the Met residue. As previous studies have suggested the Val allele as the risk allele for schizophrenia, the authors' work supports the notion that higher COMT activity in the frontal cortex might contribute towards aberrant prefrontal dopaminergic function and, in turn, to an increased vulnerability to the disorder.

3 Chen, J. *et al.* (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am. J. Hum. Genet.* 75, 807–821

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Cancer Biology

p53 inhibits the transcriptional activity of the anti-aging factor FKHRL1

Maintenance of the genome is essential for cell survival and to reduce cellular ageing. FKHRL1 is a member of the FOXO family of transcription factors, which are thought to be important for the prevention of ageing. However it is not known how these factors are regulated in response to DNA-damaging agents. You *et al.* now show that FHKRL1 is phosphorylated in a p53-dependent manner to inhibit its transcriptional activity.

The authors showed that FKHRL1 was phosphorylated in response to DNA-damaging agents in an MEF cell line containing wild-type p53, but not in cells lacking p53, showing that FKHRL1 phosphorylation requires p53. Phosphorylated FKHRL1 is known to translocate to the cytoplasm and FKHRL1 phosphorylated in a p53-dependent manner was also translocated. It was further shown that the expression of p27, which is a target for FKHRL1, is reduced. The authors showed by chromatin immunoprecipitation that FKHRL1 binding to the promoter region of its target genes was decreased only in the cell line containing WT p53.

Two kinases are known to phosphorylate FKHRL1. Of these only SGK1 was induced in the cell line containing wild-type p53, but not in the line lacking p53. To show that SGK1 is directly involved in the phosphorylation of FKHRL1, the authors introduced siRNA against SGK1 and found that it inhibited the p53-dependent phosphorylation of FKHRL1. This shows that the SGK1 kinase is essential for this phosphorylation. However further research will be required to fully understand the implications of these pathways on DNA repair and ageing.

2 You, H. *et al.* (2004) p53-dependent inhibition of FKHRL1 in response to DNA damage through protein kinase SGK1. *Proc. Nat. Acad. Sci. U. S. A.*, 101, 14057–14062

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Disease Mechanisms

Hunting for novel Huntington's disease targets

Researchers around Hans Lehrach and Erich Wanker, in collaboration with scientists at Ulm and Emory University, have recently reported a protein interaction map of the network centered around huntingtin. This is the protein responsible, in its mutated form, for the devastating inherited neurodegenerative disorder Huntington's disease. The strategy employed to generate this map involved yeast two-hybrid analysis using bait proteins derived from processes in which huntingtin has been suggested to participate.

Identified preys were used as baits in a second round of screening and interactions were confirmed by co-transformation as well as *in vitro* pull-down assays. Among the 186 protein–protein interactions detected, 165 were novel potential interactions and 32 of these could be confirmed. Furthermore, this analysis allowed functional annotation of 16 so far uncharacterized proteins and identification of GIT1, a G protein-coupled receptor kinase-interacting protein, as a promoter of huntingtin aggregation.

With respect to the pathogenesis of Huntington's disease, this work is also interesting when another recently

discovered potential mechanism for hungtin-induced neurotoxicity is considered. Researchers of Caltech and Duke University have identified activation of the IκB kinase complex by huntingtin and consequent nuclear localization of NF-κB as a contributing factor. Blocking the activation of IKK with N-terminally truncated IKKγ, or the degradation of IκB with a dominant-negative ubiquitin ligase β-transducin repeat-containing protein, reduced the toxicity of mutant huntingtin in medium-sized spiny neurons of acute striatal slice cultures. This kind of result might have been suspected from looking at the protein-interaction map of the huntingtin network, which features IKKβ as one of its nodes. This indicates that the use of such maps may, in the future, give academic research, as well as drug target identification efforts, new, productive directions.

4 Goehler, H. *et al.* (2004) A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease. *Mol. Cell* 15, 853–865

5 Khoshnan, A. *et al.* (2004) Activation of the IκB kinase complex and nuclear factor-κB contributes to mutant huntingtin neurotoxicity. *J. Neurosci.* 24, 7999–8008

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Microbiology

Finding susceptibility genes to caspofungin in yeast

Caspofungin (CAS) is a member of a new class of antifungal drugs that inhibit synthesis of an essential cell wall polysaccharide in many pathogenic fungi. In a recent paper, Markovich *et al.* analyzed a collection of *Saccharomyces cerevisiae* mutants (consisting of >4,700 individual knockout mutations) to identify genes that affect susceptibility to CAS. This information could lead to new insight about the molecular basis of CAS action and lead to further engineering of this and other antibiotics in the future.

In a screen for increased CAS sensitivity (CAS-IS) or increased CAS resistance (CAS-IR), the authors found 20 mutants that showed CAS-IS. Of these genes, 11 were involved in cell-wall and membrane function, including members of the protein kinase C integrity pathway, chitin and mannan biosynthesis genes, and ergosterol biosynthesis genes. The remaining CAS-IS mutants were involved in vacuole function and transport, transcriptional control, and unknown functions. Mutations in nine other genes were found to cause CAS-IR. Five of these genes are involved in cell-wall function and signal transduction and two are involved in vacuole function.

The authors also examined the specificity of the mutantion-altered susceptibility to CAS by testing for a change in the minimal inhibitory concentration (MIC) to other antifungals (amphotericin B, fluonazole, flucytosine and calcofluor). Seven of the 20 CAS-IS mutants and one of the nine CAS-IR mutants displayed a CAS-specific change in MIC, indicating that these genes are specifically involved in CAS susceptibility. Studies such as this will help elucidate the ways that antibiotic action can be altered at the genetic and molecular level which is invaluable in the development of new antimicrobial drugs and the battle against resistance.

6 Markovich, S. *et al.* (2004) Genomic approach to identification of mutations affecting caspofungin susceptibility in *Saccharomyces cerevisiae*. *Antimicrob. Agents Chemother.* 48, 3871–3876

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Physiology

Embryonic stem cells for broken hearts

Adult heart tissue has a limited regenerative capacity. As a consequence, cell loss or dysfunction that might occur during an infarctus is usually irreversible and can lead to progressive heart failure. Cardiac regenerative medicine, aiming to repair damaged tissue with new cells derived from skeletal myoblasts or bone marrow cells, has so far been unsuccessful.

Kehat *et al.* now show that cells derived from human pluripotent embryonic stem (hES) cells can function as pacemaker cells *in vitro* and *in vivo*. Indeed, hES integrate structurally and functionally with rat neonatal ventricular myocytes when cocultured *in vitro*. Gap-junctions are involved in this coupling, as suggested by the immunostaining of connexin-43 and connexin-45 at the interface between the two cell types, and by the abolishment of conduction by heptanol treatment.

The authors next confirm the coculture data by *in vivo* experiments where they measure, by extensive ventricular mapping, the capacity of hES cardiomyocytes to pace the heart of pigs with complete heart block. Spontaneously contracting clusters of hES cardiomyocytes, injected into the postlateral region of the left ventricle, elicit a regular, sustained and hemodynamically stable rhythm in half of the animals.

As acknowledged by the authors, this new activity could result from an indirect effect of the transplanted cells on neighbouring cardiomyocytes, because these whole-organ experiments do not allow us to map the electrical activity at the cellular level. Similarly, cell fusion as a possible mechanism of the observed results cannot be excluded, because the cardiac phenotype of differentiated hES was based solely on immunostaining. Finally, the cells were implanted in a noninfarcted tissue, which should optimize graft vascularization. Despite these limitations, this study provides evidence for the potential use of ES cells in cardiac regeneration.

7 Kehat, I. *et al.* (2004) Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat. Biotechnol.* 22, 1282–1289

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