pseudopeptides of general formula Ac-Val-DL-Ala-ψ[PO_2^--CH_2]-DL-Leu-X_{aa}'-NH_2. The ability of these pseudopeptides to inhibit human recombinant BHMT (at 100 μM) showed compound **ii** to be one of the most potent compounds, capable of an 80% inhibition of human recombinant BHMT. The phosphinic pseudopeptide inhibitors of BHMT developed in this study could be promising tools for studying the physiological function of BHMT, and further work in this area is warranted.

Val-Phe-ψ[PO_2^--CH_2]-Leu-His-NH_2

(ii)

2 Collinsova, M. *et al.* (2003) Combining combinatorial chemistry and affinity chromatography: highly selective inhibitors of human betaine: homocysteine *S*-methyltransferase. *Chem. Biol.* 10, 113–122

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Biology

Cancer biology

Survivin: antiapoptotic function by inhibiting AIF-mediated events

Inhibitors of apoptosis (IAPs) function by inhibiting caspases through their characteristic baculovirus IAP repeat (BIR). Survivin contains a single BIR domain and its expression is associated with tumour aggression. However, it remains uncertain if the anti-apoptotic properties of Survivin can be attributed to caspase inhibition.

To gain insight into the mechanism of Survivin-mediated protection in melanoma cells, Liu *et al.* [1] determined the temporal sequence of apoptotic events by employing an inducible, dominant-negative Survivin BIR mutant (T34A-Sur). The authors showed that inhibition of Survivin by induction of T34A-Sur promotes activation of caspases-3, -8 and -9. In addition, the pan-caspase inhibitor z-VAD-fmk only partially rescued T34A-Sur-induced cells from apoptosis, as determined by propidium iodide staining and flow cytometry, pointing to the existence of an apoptotic component that is caspase-independent.

It was also demonstrated that T34A-Sur is able to trigger nuclear translocation of apoptosis-inducing factor (AIF). AIF can induce caspase-independent DNA fragmentation as well as apoptotic mitochondrial events that lead to subsequent caspase activation. The authors suggest that the primary anti-apoptotic function of Survivin is likely to be the suppression of AIF function, which is a departure from the conventional view that the anti-apoptotic function of IAPs is due to caspase inhibition.

It would now be of interest to determine if the inhibition of AIF by Survivin was direct or via an intermediate. Identifying the subcellular localization of Survivin might also lend further credence to its role as a physiological regulator of mitochondrial and AIF-dependent apoptotic pathways. In addition, given the high sequence homology shared by the IAPs, one ponders the possibility that the inhibition of AIF is a general function of the IAPs or a unique property of Survivin.

1 Liu, T. *et al.* (2004) Rapid induction of mitochondrial events and caspase-independent apoptosis in Survivin-targeted melanoma cells. *Oncogene* 23, 39–48

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Reductions in apoptosis do not always enhance tumour formation *in vivo*

The importance of cell death by apoptosis as tumour-suppressive mechanism *in vivo* has been demonstrated in several ways. Selective loss of proapoptotic lesions as well as gain of antiapoptotic lesions is observed in many neoplasms. In animal models, the loss of p53, ARF, BAX or gain of Bcl-xL have been shown to cooperate with deregulated c-*myc* expression to form tumours *in vivo*, by virtue of suppressed apoptosis. This put forth the hypothesis that any significant suppression of apoptosis can cooperate with oncogenes in tumour formation.

Caspase-9 and Apaf-1 are known to have an important role in the mitochondrial pathway of apoptosis. Scott *et al.* [2] investigated their *in vivo* role in lymphomagenesis, using an IgH enhancer-driven c-myc transgene in Apaf-1–/– and caspase-9–/– mice. Due to perinatal lethality, Emicro-*myc* transgenic Apaf-1–/– or caspase-9–/– foetal liver cells were used to reconstitute lethally irradiated recipient mice. No differences were seen in rate, incidence or severity of lymphoma with loss of Apaf-1 or caspase-9, and Apaf-1 was not a crucial determinant of anticancer drug sensitivity of c-*myc*-induced lymphomas. Loss of Apaf-1 only mildly reduced the sensitivity of these cells to apoptotic stimuli for 48 hours.

This study leads us to important questions. What are the crucial differences between antiapoptotic lesions that cooperate with oncogenes *in vivo* and lesions that do not allow tumour formation *in vivo*?

Is it only the extent to which apoptosis is suppressed, complete versus partial? This is a possible explanation based on the data in this study. Or is it crucial where the lesion affects apoptosis, upstream or downstream of the mitochondria? Or do the cooperative lesions exert effects in addition to their antiapoptotic effect, for example stimulation of cell cycle progression? This study has opened the way to start addressing these questions *in vivo*.

2 Scott, C.L. *et al.* (2004) Apaf-1 and caspase-9 do not act as tumour suppressors in *myc*-induced lymphomagenesis or mouse embryo fibroblast transformation. *J. Cell Biol.* 164, 89–96

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Rad51C: a component of the Holliday junction resolvasome in mammalian cells

Despite the importance of homologous recombination for the repair of double-strand breaks in DNA, little is known about the processing of Holliday junctions (HJs) in mammalian systems. Liu *et al.* [3] have shown that Rad51C is required for HJ resolution and

that cell extracts lacking either RAD51C or XRCC3 have reduced HJ processing activity.

The authors purified HJ branch migration and resolution activity from HeLa cell extracts. Western blots were unable to detect several candidate proteins, such as RAD51, BRCA1, FEN1 or BLM. However, RAD51C was shown to co-purify with the resolvase and branch migration activities. RAD51C was depleted from the purified extract and both the branch migration and resolvase activities were reduced. RAD51C occurs as part of complexes, for example with XRCC3. Various such complexes were added to the purified extracts to see if they complemented the branch migration and resolvase activities: resolvase activity was restored by addition of any RAD51C-containing complex.

HJ resolvase activity is stimulated by the presence of ATP. The authors tested mutants of RAD51C for complementation of activity. RAD51C containing a point mutation within the ATP-binding domain had reduced activity, whereas a construct lacking the RAD51C C-terminal domain had no activity. Thus, RAD51C is required for HJ resolvase activity in mammalian cells.

HJ branch migration and resolvase activity were purified from CHO cell extracts defective in XRCC2, XRCC3 or RAD51C. Only the extracts defective in XRCC3 and RAD51C were found to have reduced resolvase activity, suggesting that RAD51C and XRCC3 are required for HJ processing.

To date, no resolution or branch migration activity has been seen by recombining individually purified proteins. It is likely that additional factors, and also specific post-translational modifications, are required for these activities. Clearly, more research is required into this process because it is so fundamental to the prevention of cancer.

3 Liu, Y. *et al.* (2004) Rad51C is required for Holliday junction processing in mammalian cells. *Science* 303, 243–246

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Targets and Mechanisms

Not-quite-so-selective COX-2 inhibitors

Selective COX-2 (cyclooxygenase-2) ligands show similar efficacy to marketed non-steroidal anti-inflammatory drugs (which act at both COX isoforms) but with a reduced side-effect profile, in particular in terms of gastro-intestinal (GI) effects. Marketed drugs such as celecoxib (Celebrex), valdecoxib (Bextra) and rofecoxib (Vioxx) are used to treat pain and inflammatory disease associated with arthritis.

Supuran *et al.* [4] have now demonstrated that the sulfonamide containing COX-2 inhibitors (valdicoxib and celecoxib) bind to carbonic anhydrases (CA) *in vitro* (IC_{50} values of sub-50 nM at human CAs II and IX). These binding affinities are comparable with those of marketed CA inhibitors such as methazolamide and dichlorophenamide, the latter having been available for over 45 years. Other COX-2 inhibitors that do not posses the sulfonamide group have no activity at CAs. Analysis of crystal structures have shown that the sulfonamide group interacts with the catalytic zinc unit of CA's whereas a determination with SC-558 (a close analogue of celecoxib) has demonstrated that the sulfonamide group interacts with His-90, Gln-192 and Leu-352.

These *in vitro* binding properties can be translated into an *in vivo* effect. CA inhibitors are marketed for the treatment of glaucoma; both CA II and CA IX are found in the glaucomatous eye and their inhibition leads to a lowering of intraocular pressure (IOP). Both celecoxib and valdecoxib have now been shown to significantly reduce IOP in hypertensive rabbits, whereas COX-2 inhibitors lacking a sulfonamide group have no effect.

CAs (and in particular CA IX) are upregulated in several cancer cell lines, which could provide a rationale for the investigation of COX-2 inhibitors in this disease area.

4 Supuran, C.T. (2004) Unexpected nanomolar inhibition of carbonic anhydrase by COX-2-selective celecoxib: new pharmacological opportunities due to related binding site recognition. *J. Med. Chem.* 47, 550–557

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Just folding back: convergence of viral fusion mechanisms

Enveloped virus infection begins with viral-cellular membrane fusion and is mediated by class I or II viral fusion proteins.

For class I fusion proteins, like haemagglutinin from influenza A virus and gp41 from HIV, conformational changes in viral membrane fusion is well-documented: there are at least three conformations (pre-fusion native, intermediate pre-hairpin and stable post-fusion) and their two heptad repeat regions have different conformations in different states.

For class II viral fusion proteins, however, it was not clear how the conformational changes contribute to the fusion, until two publications of the crystal structures of dengue virus E protein and Semliki Forest virus, in their post-fusion conformations [5,6]. In both cases, the proteins form homodimers with three structural domains that lie flat on the virus surface, unlike class I proteins, which form 'projections' on the surface. The structures show that, under low-pH conditions, the homodimers change into homotrimers with these three domains intact but rearranged into a different conformation; this might represent the post-fusion conformation. The most important change is that domain III refolds towards the cellular membrane direction, pulling the viral and cellular membrane into proximity as domain III is linked to the transmembrane domain through the stem region.

The authors propose that there must be an intermediate conformation after receptor-binding, which triggers the fusion loop to reach the cellular membrane, in a similar way to the class I fusion proteins. This intermediate conformation works as a bridge for the transition from native conformation into post-fusion conformation.

In conclusion, the new crystal structures on the post-fusion conformation of class II viral fusion proteins provide evidence of the convergence of the molecular mechanism of virus fusion. These studies provide a new drug target for class II virus fusion inhibition as successfully used in class I viruses.

5 Modis, Y. *et al.* (2004) Structure of the dengue virus envelope protein after membrane fusion. *Nature* 427, 313–319
6 Gibbons, D.L. *et al.* (2004) Conformational change and protein-protein interactions of the fusion protein of Semliki Forest virus. *Nature* 427, 320–325

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Microbiology

Bacteriophage evolution: nature's own antimicrobial HTS

Bacteriophage genomes could serve as an evolutionary 'screened' source for genes encoding proteins with specific cellular targets in bacteria. These targets might be further investigated for bacterial growth inhibition, and ultimately low-molecular weight compounds binding to the targets could be developed for testing as novel antibiotics. At present, there is a great need for rapid development of novel classes of antibiotics to treat patients infected with *Staphylococcus aureus*.

Liu *et al.* [7] sequenced 26 genomes from bacteriophages infecting *S. aureus* and screened for gene products that inhibited growth: 31 novel protein families were identified. The bacterial targets of growth-inhibiting proteins were identified. The interaction between one such phage protein (phage 77 Orf104) and its bacterial target, DnaI was validated by yeast two-hybrid assays, ligand blots, surface plasmon resonance, and time-resolved FRET.

Subsequently, the *dnaI* gene was shown to be essential for *S. aureus* using an inducible promoter replacement system, and 77ORF104 was shown to inhibit DNA synthesis when expressed in *S. aureus*. The authors hypothesized that a small-molecule compound that can inhibit the interaction between DnaI and 77ORF104 could also inhibit bacterial growth. 125,000 small-molecule compounds were screened for inhibition of the DnaI-77ORF104 interaction using a fluorescence-based assay: 11 were shown to inhibit growth with minimal inhibitory concentrations of $<16\ \mu g\ ml^{-1}$.

This study shows how bacteriophage genomics can be used to search for essential bacterial targets that can be used for antibiotic development. By exploiting the evolutionary 'pre-screening' for antimicrobial compounds that continuously takes place in the bacteria–bacteriophage interaction, the identification of relevant targets for subsequent screening can be greatly facilitated.

7 Liu, J. *et al.* (2004) Antimicrobial drug discovery through bacteriophage genomics. *Nat. Biotech.* (e-pub ahead of print; http://www.nature.com)

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Genomics and Proteomics

RNAi-based target discovery is paying off in the clinic

Until recently, target validation relied mostly on the analysis of genetic knockout mice. 'Cleanly' wiping out single genes is an attractive model for simulating the action of the 'perfectly' selective, potent drug, before initiating the costly drug-screening process.

However, knockout mice are difficult to make and there is no opportunity to study dose-response effects. The disease context in which we need to test the mutation is not always obvious, and if the gene is essential for embryonic development, there might be no mouse to study at all.

The field of target discovery changed dramatically with the finding that short, double-stranded RNAs (siRNA or RNAi) enable researchers to knock down the expression of nearly any gene. Much of the current efforts [9,10], focus on ways to generate RNAi-delivering tools to probe thousands of druggable targets in cell-based assays. The two papers describe clever methods to construct such libraries from 'random' cDNAs.

In another recent paper [11], it was demonstrated that screening such libraries to discover 'opportunistic' targets is highly successful. Brummelkamp *et al.* used a library to deliver sets of siRNAs against 50 human de-ubiquitinating enzymes in various cancer-related cellular assays. These enzymes are known to regulate protein stability and to have a role in signaling. One of the candidates, CYLD, was identified as a key regulator of the NF-κB pathway. Skin tumours were known to develop in patients with a mutation in CYLD, but the mode of action for this oncogene was unknown. Based on the team's observations, these cylindromatosis cancer patients are currently being tested in the clinic with drugs that are known to block NF-κB activation.

We can safely predict that these RNAi screens will yield many other exciting disease targets in the near future.

9 Sen, G. *et al.* (2004) Restriction enzyme-generated siRNA (REGS) vectors and libraries. *Nat. Gen.* 36, 183–189
10 Shirane, D. *et al.* (2004) Enzymatic production of RNAi libraries from cDNAs. *Nat. Gen.* 36, 190–196
11 Brummelkamp, T.R. *et al.* (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-κB. *Nature* 424, 797–801

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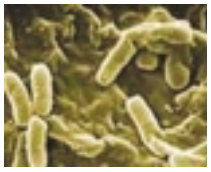
The secondary 'language' of *Pseudomonas aeruginosa*

Quorum sensing (QS) is a chemical signaling system in Gram-negative bacteria, where cells use self-generated metabolites as signal molecules to sense the cell population. *N*-acyl-*L*-homoserine lactones (AHLs) are the most frequently used extracellular molecules.

Pseudomonas aeruginosa is the best-studied model of AHL-mediated QS because two separate pairs of autoinduced AHL synthase and transcriptional regulator, LasR/I and RhlR/I, are involved in modulating the expression of multiple genes. In addition, a distinct molecule – 3,4-dihydroxy-2-heptylquinoline (PQS) – serves as a link between the regulatory networks of Las and Rhl. PQS belongs to a family of antimicrobial compounds, 4-hydrooxy-2-alkylquinolines (HAQs), produced by *P. aeruginosa*.

Déziel *et al.* [8] first employed LC–MS to separate and deduce the five structural classes of HAQ congeners. The proposed transcriptional regulator mvfR mutant was incapable of producing any HAQs.



Scanning Electron Micrograph of *Pseudomonas aeruginosa*. CDC/Janice Carr.

Comparative gene expression profiling indicated that genes in the phnAB operon, including *pqsA–E*, were controlled by the mvfR and subsequent genetic inactivation established catalytic roles for proteins in the HAQ pathway, in which 4-hydroxy-2-heptylquinoline (HHQ) produced as a major congener is the direct precursor of PQS.

Finally, the authors demonstrated that in the bacterial community PQS production also relies on the HHQ available in the extracellular milieu. As another N-oxide HHQ derivative has the major antimicrobial activity of all HAQs, whereas PQS does not, they probably reflect two different messages conveyed among the cells.

This work identifies and characterizes a second signaling molecule and its link to the primary QS system of *P. aeruginosa* that commonly causes nosocomial chronic infections of immunocompromized patients. Particularly, HHQ and PQS were found to be significant in the lungs of cystic fibrosis patients. The HHQ biosynthetic pathway and its regulation should represent an array of potential drug targets for treating this disease.

8 Déziel, E. *et al.* (2004) Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2- heptylquinoline in cell-to-cell communication. *Proc. Natl. Acad. Sci. U. S. A.* 101, 1339–1344

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Business

Collaborations

BioXell and ProSkelia collaborate in osteoporosis

BioXell SpA (http://wwwbioxell.com) and Proskelia SAS (http://www.proskelia.com) have announced an R&D collaboration for drug candidates based on vitamin D3 analogues for the treatment of osteoporosis and secondary hyperparathyroidism (HPT).

BioXell's broad vitamin D3 platform has enabled them to discover several novel compounds, some of which fall outside the company's key focus areas of urology and inflammatory diseases. ProSkelia have concentrated its research efforts on the development of novel treatments for osteoporosis and other bone diseases.

Roland Baron, Founder and CSO of ProSkelia, said: 'The compounds involved in this collaboration are core to our business and will augment our existing pipeline of products aimed at this therapeutic field.' Founder and CEO of BioXell, Francesco Sinigaglia, commented: 'The coupling of BioXell's expertise in vitamin D3 with ProSkelia's discovery and development capabilities… is a powerful combination designed to result in the expedited discovery of patentable clinical drug candidates.'

Archemix and JnJ: GPCR targets and aptamers

Archemix (http://www.archemix.com) have announced a target validation collaboration with Johnson & Johnson Pharmaceutical R&D (http://www.jnj.com), which will focus on validating G protein-coupled receptor (GPCR) targets.

Errol De Souza, President and CEO of Archemix, said: 'Archemix is enthusiastic about the opportunities for using aptamers for target validation. … Archemix will be able to leverage the aptamer technology generated in these collaborations for use within its aptamer therapeutic programs.'

Archemix is a biopharmaceutical company focused on discovering and developing aptamers as a new class of directed therapies for a wide range of disease areas.

Chemogenomics collaboration: Iconix and Abbott

Iconix Phamaceuticals (http://www.iconixpharm.com) have entered a research collaboration with Abbott Laboratories (http://www.abbott.com) to apply Iconix's chemogenomics technology in Abbott's drug discovery and development efforts.

Iconix's DrugMatrix® system – the world's largest source of information on the genomic effects of drug and chemical treatments – and library of Drug Signatures™ will be used, as well as technology to identify biomarkers for the clinical development and commercialization of Abbott's therapeutic products.

James B. Summer, divisional VP, Advanced Technology, Drug Discovery at Abbott, said: 'Iconix's technology will enhance our understanding of potential drug candidates and can be applied throughout Abbott's R&D process.' Jim Neal, CEO of Iconix, commented: 'We are confident that in the near future chemogenomics will become a key tool in drug discovery and development and an integral part of regulatory submissions.'

Business was written by *Joanne Clough*

People

Appointments

Affibody appoints new Chief Scientific Officer

Affibody (http://www.affibody.com), a Swedish company focused on the areas of bioseparation, proteomics and bioinformatics, has announced the appointment of Lars Abrahmsén as CSO, replacing one of the company's founders, Stefan Ståhl, who has returned to his academic professorship. Ståhl will remain as a scientific advisor to the company.

Abrahmsén joins Affibody from Biovitrum, where he was senior project team leader.

Torben Jørgensen, CEO of Affibody, said: 'Having Lars Abrahmsén in our management team… is highly beneficial for us. This recruitment supports our progression of development of protein therapeutics using Affibody molecules.'

Abrahmsén, who is recognized for his work on protein pharmaceuticals, said: 'Affibody's technologies hold very

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