design of gene-targeted drugs [5–7]. The promise of this technology rests on the expectation that synthetic 'gene-blocking' oligomers, which have a sequence that is complementary to corresponding mRNA or pre-mRNA targets, will become a generic therapy of the future for various diseases. After common oligonucleotides and their modified analogues, a third generation of antisense oligomers – the artificial DNA and RNA mimics – is emerging, with the peptide nucleic acid (PNA) oligomers showing significant promise [8].

Recent studies performed at ISIS Pharmaceuticals (http://www.isip.com) with the murine cellular receptor CD40, which has a key role in immune response, have demonstrated that specific downregulation of protein expression can be efficiently achieved with the PNA antisense inhibitor ISIS208529; this molecule targets the exon 6 splice junction within the primary CD40 transcript [9]. When delivered to murine cells (primary macrophages and lymphoma B-cells) by electroporation, binding of ISIS208529 interferes with pre-mRNA splicing and results in the accumulation of a defective protein that lacks the transmembrane domain. Conjugation of ISIS208529 with oligolysine yields an even more potent antisense drug, ISIS278647, which is effective in murine cells via 'free uptake' (i.e. without transfection or electroporation). Importantly, cells that have been treated with PNA inhibitors exhibit a decrease in the CD40-mediated production of the cytokine interleukin-12 (IL-12) as a result of inhibition of the CD40-signalling pathway.

These results show that antisense PNA oligomers can be employed as new immunomodulatory agents. Given that just a few antisense drugs have entered clinical trials [10], and with only one such drug currently approved for clinical use (antiviral vitravene or fomivirsen; ISIS2922), these novel drug candidates are valuable additions to the small antisense family.

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Biology

Microbiology

Novel *Streptococcus pyogenes* exotoxin disrupts cytoskeleton

Exotoxins with ADP-ribosyltransferase (ADPRT) activity are produced by many bacterial pathogens. ADPRTs transfer ADP-ribose from β-NAD^+ onto host target proteins such as transcription factors, signaling molecules, and cytoskeletal proteins and thereby interfere with their function. In the important human pathogen *Streptococcus pyogenes* there are two known ADPRTs (GAPDH and SPN), but no cellular targets have been identified.

Coye *et al.* identified a novel putative ADPRT, SpyA, which is present in several genomes of various serotypes [1]. Recombinantly expressed SpyA, but not SpyA mutated in a putatively catalytic glutamic acid residue, has NAD-glycohydrolase activity and ribosylates poly-L-arginine. Besides auto-ribosylation, SpyA ribosylates several proteins when incubated with cellular extracts. Two-dimensional electrophoresis and mass spectroscopy identified the cytoskeletal proteins actin, vimentin and tropomyosin as SpyA targets. Expression of SpyA in HeLa cells and fluorescence microscopy demonstrated that actin microfilaments were disrupted. The authors hypothesize that this modification could interfere with phagocytosis of the bacteria by professional phagocytes. It is still unclear how SpyA enters host cells, but the authors speculate that cytolysin mediated translocation, which is crucial for delivery of SPN, could be involved. Another possibility not mentioned in this paper is that SpyA could be expressed by intracellular bacteria within the phagocytes and contribute to survival by cytoskeletal rearrangements.

This study describes a novel ADP-ribosyltransferase and for first time identifies cellular targets for an ADPRT from *S. pyogenes*. Even though there are still several questions to be answered regarding *in vivo* expression and cellular translocation/intracellular expression, this report indicates that SpyA and other ADPRTs could be important for the molecular pathogenesis of *S. pyogenes.*

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A bit of Lov for HIV-1 patients

HIV-1 entry into and exit from target cells requires adequate cholesterol levels in host and viral membranes. Protein co-aggregation is also needed at the host cell surface: CD4 and chemokine receptors for entry, Gag and gp160 for budding. The HIV-1 infection process induces receptors clustering with lipid rafts, which necessitates actin cytoskeleton rearrangements. Rho is suspected to play a key role during this reorganization process.

Del Real *et al.* now provide evidence that statins prevent HIV-1 infection in cultured primary cells, in animal models and in chronically infected individuals [2]. Statins, currently used to treat hypercholesterolemia, inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. HMG-CoA reductase produces mevalonic acid, a precursor for cholesterol.

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They first show that human PBMC pretreated with lovastatin (Lov), which belongs to the statins family, are protected from HIV-1 infection *in vitro*. Single round infections were performed with X4 (NL4-3) or R5 (BaL) HIV-1 strains. Infection inhibition is reversed by coincubation of cells with L-Mev, the product of HMG-CoA reductase. They next show that Lov injections of SCID-hu-PBMC mice (SCID mice reconstituted with human PBMCs), before HIV-1 NL4-3 challenge, block HIV-1 replication *in vivo*. Further *in vitro* experiments suggest that Lov inhibits HIV-1 entry into target cells, at least in part, by preventing Rho activation.

Finally, the authors investigate the use of statins in six chronically infected HIV-1 patients with a stable viral load for at least six months. One month of Lov treatment as sole therapy clearly reduces viral RNA loads in all individuals. RNA levels rebound when the treatment is discontinued. This study highlights the antiretroviral potential of statins to treat the AIDS pandemic.

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Cancer Biology

NF-κB and AP-1: making the connection

NF-κB and AP-1 are two transcription factors key to the control of developmental, physiological and pathological processes, such as embryogenesis, the inflammatory response and tumour formation, respectively. In this, they share many target genes that they regulate cooperatively. So far, there has been only fairly circumstantial data suggesting that there exists a direct connection between NF-κB and AP-1, in which the former transcription factor appears to be able to modulate the activity of the latter. One of the most striking pieces of such evidence is the finding that AP-1-dependent transcription of the VEGF gene is regulated by NF-κB, without there being a binding site for this transcription factor present in the promoter.

A recent article by Fujioka *et al.* [6] presents direct evidence that NF-κB can stimulate AP-1 activity in response to doxycycline and, to a lesser extent, serum. The authors suggest that this occurs via induction of elk-1 transcription by NF-κB. Elk-1 is a transcription factor activated by MAP kinase and required for the expression of genes encoding components of AP-1, such as c-fos. Expression of a transdominant IκB mutant or IKK knockout resulted in decreased Elk-1 levels, delayed (serum-induced) or completely suppressed (doxycycline-induced) c-*fos* and *fosB* expression and inhibition of inducible AP-1 activity. Although Elk-1 levels were greatly reduced in both IKK-1 and IKK-2 null cells, only the latter showed significant inhibition of c-*fos* and *fosB* expression, pointing to additional factors required for the transcription of these genes being adversely affected by the absence of IKK-2.

In this context it is interesting to note that an inhibitor of IKK-2, SPC-839 [7], has been reported to inhibit PMA/PHA-stimulated NF-κB – as well as AP-1-dependent reporter gene expression [8]. However, because this is not a general feature of potent and supposedly selective IKK-2 inhibitors, inhibition of this kinase by SPC-839 appears unlikely to be responsible for this effect.

Glycobiology

Expressing proper glycoproteins in milk

Many human proteins are in great demand now as potent biotherapeutics and the most convenient way of obtaining them is recombinant expression. But this approach is problematic for human glycoproteins that are active only when their polypeptide core is properly conjugated with the complex net of polysaccharides, mostly N- and O-glycans. The issue is that bacterial hosts are incapable of protein glycosylation, whereas yeasts can do this only in the form that differs from humans.

As a result, those human proteins that have to be glycosylated are obtained from these two major suppliers of recombinant polypeptides as either inactive molecules or active drugs that cause unwanted immune responses. Use of mammalian cell cultures is an option but rather expensive and not always efficient. To this end, the mammary glands of transgenic animals represent an attractive opportunity [3]. These live bioreactors can yield the high-level expression of glycoproteins, with close to human glycoforms.

Recently, human C1 inhibitor (hC1INH), a therapeutic N,O-glycoprotein with a growing number of clinical applications, has been expressed in the milk of transgenic rabbits [4]. Although the glycan analysis revealed that the rabbit milk-expressed hC1INH contained a broad array of different N-glycans, in contrast to its human serum original, it was found later that the major N-glycan structures of recombinant protein in pooled rabbit milk are similar to native ones [5]. As to O-glycans, they showed the usual glycosylation pattern [4].

Accordingly, large quantities of thus humanized recombinant hC1INH were isolated for preclinical and clinical studies featuring highly consistent glycan profiles and monosaccharide compositions. It is projected that kilograms per year of this drug could be obtained using milking rabbits to put it on the market next year.

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An unfamiliar consequence of *BRCA1* inactivation

Familial *BRCA1*-associated breast cancers display a distinctive histopathology and profile of gene expression. Loss of the cell cycle regulator, p27, is a common finding, yet the functional relationship between *BRCA1* and p27 has not been well defined.

Researchers in Melbourne, Australia, have recently created a transgenic mouse expressing a dominant negative allele of *BRCA1* in the mammary gland [9]. This mouse was found to have delayed mammary development. Contrary to expectation, it also displayed elevated levels of p27 and reduced proliferative capacity. *In vitro* studies confirmed that acute loss of *BRCA1* did indeed augment p27 expression. This was not due to altered proteosomal degradation, but probably reflects an increased level of the mRNA.

In contrast, expression of the *BRCA1* transgene on a p27 heterozygote background resulted in normal mammary gland development. However, there was an associated increase in the number of cells in S phase. Surprisingly, p27 was the only cell cycle regulator affected by *BRCA1* inactivation, suggesting that this molecule is central to the observed phenotype.

Extrapolating these results to humans, it appears that p27 could initially accumulate in response to germline *BRCA1* mutations. This would presumably counteract the effects of *BRCA1* inactivation by inducing growth arrest. However, subsequent events favour reduced p27 and an increased proliferation rate. Loss of p27 is therefore likely to be a prerequisite for *BRCA1*-associated tumourigenesis. It is possible that in future, drugs designed to maintain elevated levels of p27 might block this effect, allowing prevention, rather than cure, in patients harbouring familial *BRCA1* mutations.

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Business

Collaborations

Peakdale and Cyprotex collaborate on drug-like screening compounds

Peakdale Molecular (http://www.peakdale.co.uk) and Cyprotex (http://www.cyprotex.com) have announced their collaboration in the application of ADME technology to maximize the drug-like properties of Peakdale's unique screening compounds. Cyprotex will use its CloeScreen™ HTS technology to evaluate the ADME properties of approximately 8500 novel lead-like compounds developed and synthesized by Peakdale.

The ADME data generated from the collaboration will be used to develop predictive models that will form the basis for the enhancement of the design of novel compounds. The provision of compounds with enhanced ADME profiles enables Peakdale to offer its clients superior starting points from which to discover new drugs, in addition to making substantial improvements in the efficiency of subsequent optimizations.

'Consistent, high quality compound data are essential for efficient drug discovery because it enables you to predict the potential of new compounds before they've even been synthesized,' commented David Leahy, Cyprotex's Chief Scientific Officer. 'Combining our predictive models with Peakdale's unique chemistry will offer drug-like leads that will be ready for trials earlier and offer a greater chance of success.'

Gareth Jenkins, Peakdale's Business Development Manager, added: 'Our customers already benefit from our novel chemistry expertise. Now they can benefit further from better quality compounds, faster development times and reduced risk from failure in clinical trials.'

Strand and Bio-Rad

Strand Genomics (http://www.strandgenomics.com) has entered into an agreement with Bio-Rad (http://www.bio-rad.com) with a view to integrating Strand Genomics' predictive human pharmacokinetic models into Bio-Rad's KnowItAll® platform for *in silico* ADME–Tox assessment. Strand's ADME-Predict™ uses the chemical structure of potential drug candidates to generate predictions for human pharmacokinetic parameters that are applicable to drug discovery, including bioavailability, volume of distribution and protein binding.

Kas Subramanian, Strand's Chief Scientific Officer, remarked: 'We are pleased to combine this expertise and technology along with Bio-Rad's award winning KnowItAll™ platform. This combined expertise will allow medicinal chemists and pharmacologists to design and optimize leads and candidates that have the greatest chance of succeeding in the clinic.'

'We look forward to working with Strand Genomics in moving forward to accelerate drug discovery,' added Gregory Banik, General Manager, Bioinformatics Division at Bio-Rad. 'The addition of these predictors emphasizes Bio-Rad's continued commitment to offer the most comprehensive ADME–Tox prediction portfolio to the drug discovery community.'

Strand Genomics is a Bangalore-based life sciences informatics company focusing on software for drug discovery and development. Strand's core strengths lie in data mining and analysis, knowledge management and workflow processes to develop its products and provide high-end technology consulting services.

GenData and Battelle to collaborate on the identification of predictive diagnostics for COPD

GenData (http://www.gendata.org) and Battelle (http://www.battelle.org) have entered into a collaborative study for the

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