contain a reactive peptide bond (P_1–P_1') located between the Arg5 and Ile6 residues. X-ray crystallography has demonstrated that 13 amino acid residues (residues 1–7, Glu9 and residues 25–29) of CMTI-I are directly involved in its interaction with trypsin. These inhibitors potentially have therapeutic applicability in the treatment of cystic fibrosis and pancreatic disease, as well as in antiretroviral therapy approaches. Recently, Kazmierczak *et al*. [4] investigated the *N*-terminal segment of CMTI, which is directly involved in the interaction with bovine β-trypsin, as a possible template to design low-molecular-mass proteinase inhibitors. A solid-phase library of 2016 decapeptides in mixtures was synthesized on TentaGel S Ac with attached Fmoc-Gly resin (RAPP Polymere; http://www.rapp-polymere.com). Deconvolution identified active peptide constituents. The decapeptides were screened against bovine β-trypsin and bovine α-chymotrypsin. One of the most potent deconvoluted compounds isolated was **iii,** which possessed a K_a of 1.1 x 10^8 M^{-1} against bovine β-trypsin. Although this analogue is three- to fourfold less active than wild-type CMTI inhibitors, this type of analogue has a lower molecular weight and fewer conformational constraints than wild-type inhibitors. Thus, these decapeptides represent promising starting points for the optimization of these low-molecular-weight serine proteinase inhibitors and this work warrants further investigation.

[Homoarginine-Val-Gly-Pro-Arg-Ile-Leu-Met-Homoarginine-Gly]CMTI-$III_{1–10}$

(iii)

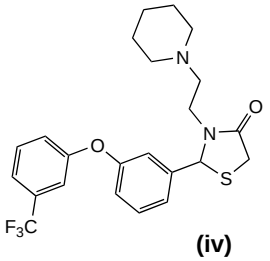
3 Wieczorek, M. *et al*. (1985) The squash family of serine proteinase inhibitors. Amino acid sequences and association equilibrium constants of inhibitors from squash, zucchini, and cucumber seeds. *Biochem. Biophys. Res. Commun.* 2, 646–652

4 Kazmierczak, K. *et al*. (2003) Selection of low-molecular-mass trypsin and chymotrypsin inhibitors based on the binding loop of CMTI-III using combinatorial chemistry methods. *Biochem. Biophys. Res. Commun.* 310, 811–815

Sodium channel agonists

Chronic pain in humans represents a disease for which there is currently a significant unmet medical need. According to statistics from the American Society of Anesthesiologists (http://www.asahq.org), back pain disables five million people per year in the US. Another 66 million people experience pain related to arthritis, 40 million people have recurrent severe headaches and 90% of terminal cancer patients suffer from severe pain associated with the disease. Prescribed non-steroidal anti-inflammatory agents, over-the-counter pain medication or, in the most severe cases, opioids are the front-line therapies currently used to treat the condition. Most of these treatments have undesirable side effects associated with their application. The discovery of a broad spectrum, highly effective analgesic agent that is also devoid of side effects is a common goal within the pharmaceutical industry.

Ion channels that regulate the excitability of sensory neurons might represent plausible drug targets for the treatment of chronic pain. Specifically, voltage-gated sodium channels are attractive targets because they are essential for the inhibition and propagation of neuronal impulses. Furthermore, they play a crucial role in determining the activation threshold and firing patterns in injured primary afferent neurons. Thus, optimization of sodium channel antagonist pharmacology as an approach to the discovery of new analgesic agents has been a recent goal for Sun *et al*. [5]. A library of 91 thiazolidinone compounds was synthesized as singletons in solution. For the measurement of K_i values of synthesized compounds, oocytes were held at a holding potential of –120 mV followed by a four second depolarizing step ('pre-pulse') to –10 mV in which the maximum current was elicited. At the end of this depolarization step, nearly all of the channels would be in the inactivated state. Next, a 10 ms hyperpolarizing step was performed to remove some channels from the inactivated state. A final depolarizing step ('test pulse') was used to assay the sodium current following this prolonged depolarization. Sodium currents were measured at this test pulse before and after drug application. One of the most potent compounds found was **iv,** which displayed a K_i of 90 nM. This work has produced potent and novel sodium channel antagonists that have improved pharmaceutical and pharmacological properties and further work in this area is warranted.



(iv)

5 Sun, Q. *et al*. (2003) Parallel synthesis of a biased library of thiazolidinones as novel sodium channel antagonists. *Comb. Chem. High Through. Screen.* 6, 481–488

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Biology

Microbiology

Streptococcus pyogenes IL-8 protease is inhibited by pheromone

The strictly human pathogen *Streptococcus pyogenes* causes relatively mild localized upper respiratory tract and skin infections, but occasionally causes severe invasive and/or tissue-destroying infections with high mortality. *S. pyogenes* has evolved several strategies to evade the immune response by binding to plasma proteins and immune regulators, as well as immuno-modulating enzymes interacting with the immune system [1].

Hidalgo-Grass *et al*. [2] analyzed *S. pyogenes* isolates and tissues from patients with necrotizing infections. Infected tissue contained numerous bacteria, but no infiltrating neutrophils. Bacteria were shown to release a protease activity that degrades the human chemokine interleukin 8 (IL-8), which is important for neutrophil recruitment. In a mouse model of soft-tissue infection the bacteria produced fatal necrotic infections with reduced neutrophil infiltration. Furthermore, the strains were mutated in *silCR*, encoding the pheromone peptide

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SilCR. Purified SilCR inhibited production of IL-8 protease activity *in vitro* and restored neutrophil migration, thereby rescuing mice infected with original isolates and well as heterologous strains.

This study describes a new immuno-modulating enzymatic activity (IL-8 protease) of *S. pyogenes*, but the exact nature of the enzyme is unclear and needs to be further addressed. Nevertheless, the study clearly demonstrates that peptide pheromones have therapeutic potential in serious necrotizing infections by regulating bacterial protein expression.

1 Collin, M. and Olsén, A. (2003) Extracellular enzymes with immuno-modulating activities: variations on a theme in *Streptococcus pyogenes*. *Infect. Immun.* 71, 2983-2992

2 Hidalgo-Grass, C. *et al.* (2004) Effect of a bacterial pheromone peptide on host chemokine degradation in group A streptococcal necrotising soft-tissue infections. *Lancet* 363, 696-703

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Genome plasticity in *Streptomyces*

Streptomyces bacteria are important organisms not only in the natural environment but also in the pharmaceutical and agricultural industries where their secondary metabolites are harvested and used for several different purposes. Studying the genomes of different *Streptomyces* strains will provide valuable insight and aid our understanding of these microbes at the molecular level, with the possibility of better genetic engineering of these organisms for beneficial purposes.

The central portion of the *Streptomyces coelicolor* 8.7 Mb linear genome encodes conserved housekeeping genes essential for growth, but the ends (or 'arms') the genome are highly dynamic and vary greatly in structure and gene content. In a recent paper by Weaver *et al*. [3], the chromosomes of several laboratory derivatives of *S. coelicolor* strain A3(2) were found to have 1.06 Mb of inverted repeat sequences at their termini (so-called long-terminal inverted repeats or L-TIRs). This was in marked contrast to the sequenced *S. coelicolor* strain M145, which had TIRs only 22 kb in size and was thought to be genetically similar to the other derivatives containing L-TIRs.

As many of the *S. coelicolor* strains with short TIRs were exposed to ultraviolet (UV) or X-ray light during laboratory manipulation, the authors hypothesized that X-ray and/or UV irradiation could have induced truncation of the L-TIR to the 22 kb length, and they confirmed this experimentally. The L-TIRs are the longest terminal sequences so far reported for an actinomycete, and the authors have proposed that they represent the chromosomal structure of the original soil isolate of *S. coelicolor* A3(2).

3 Weaver, D. *et al*. (2004) Genome plasticity in *Streptomyces*: identification of 1Mb TIRs in the *S. coelicolor* A3(2) chromosome. *Mol. Microbiol*. 51, 1535–1550

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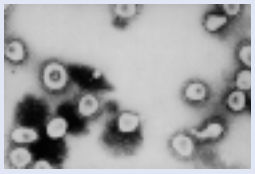
Virology

SARS: structures from components of the virus are beginning to emerge



Severe acute respiratory syndrome (SARS) is a member of the coronavirus family. It has a complex genetic organisation and so the replication cycle is poorly understood. Family members have a large replicase gene that is processed into a number of components that form a replication/transcription complex. This complex includes nonstructural proteins (nsp) of unknown function. Egloff *et al*. [6] have solved the structure of one of these proteins, nsp9, and shown that it binds to single-stranded RNA (ssRNA).

Nsp9 is dimeric, with each monomer containing an β-helix and a barrel of 7 β-strands. The sequence has no known homologues, but the β-barrel is similar to the 5-stranded β-barrel of the oligosaccharide/oligonucleotide-binding (OB) fold, mainly found in nucleic acid-binding proteins. If nsp9 and the OB fold are superimposed the binding site would be within the dimer interface of nsp9, suggesting that nsp9 represents a new variant of the OB fold.

The authors showed that nsp9 does bind to ssRNA by examining tryprophan fluorescence quenching in the presence of ssRNA or ssDNA oligonucleotides. Nsp9 bound with a similar affinity to both RNA and DNA, probably via a negatively charged surface on the dimer, suggesting that it is not sequence specific.

A lot of work is now going into solving the structures of the proteins encoded by the SARS coronavirus genome to help develop antiviral drugs that could prevent future epidemics. Single-stranded nucleotide- binding proteins encoded by viruses are frequently essential, so nsp9 is potentially a good target.

6 Egloff, M-P. *et al*. (2004) The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA binding subunit unique in the RNA virus world. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3792–3796

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Cancer

Loss of c-*Myc* confers selective resistance to apoptosis

The nuclear proto-oncogene c-*Myc* plays and important role in proliferation, differentiation and apoptosis. Overexpression of c-*Myc* in the presence of survival factors leads to proliferation and to apoptosis in the absence of survival factors. Grassilli *et al.* now confirm the importance of c-*Myc* in apoptosis by showing that c-*Myc*-null cells are resistant to apoptosis by several anticancer agents [4]. Doxorubicin and etoposide-induced apoptosis is impaired in c-*Myc* null cells, whereas camptothecin-induced cell death occurs normally. The authors show that p53 and its proapoptotic effector molecule Bax accumulate in c-*Myc* null cells exposed to doxorubicin, but fail to die.

This important finding shows that c-*Myc* does not merely induce apoptosis by inducing p53, but that c-*Myc* itself is needed for final execution of death. This confirms the early studies of c-*Myc*-induced apoptosis in rat-1 fibroblasts, which are effectively killed by overexpression of c-*Myc* in absence of survival factors. The authors show that complete protection can from doxorubicin-induced apoptosis in wild-type cells can only be achieved when

caspases, serine proteases and cytochrome *c* release are blocked simultaneously and suggest c-*Myc* is essential to all pathways.

Future research will be needed to see if these findings can be extrapolated to other oncogenes and determine whether loss of functions will confer resistance to certain subsets of anticancer drugs. The current data have implications for the clinical setting and could change the choice of drugs for treatment of tumors driven by a MYC family member.

4 Grassilli, E. *et al*. (2004) Loss of Myc confers resistance to doxorubicin-induced apoptosis by preventing the activation of multiple serine protease-and caspase-mediated pathways. *J. Biol. Chem*. DOI 10.1074/jbc.M31353220, (E-pub ahead of print; http://www.jbc.org/)

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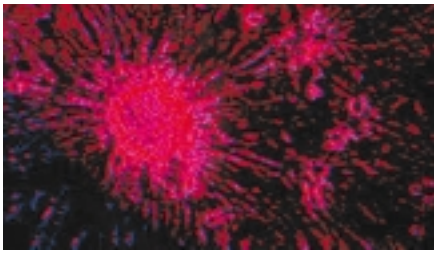
Lessening the resistance

Tumor cells can develop a very effective resistance mechanism to multiple antitumoral drugs, causing the failure of



cancer chemotherapy treatments. The multiple drug resistance (MDR) is due to either the cell-surface overexpression of transmembrane efflux pumps, P-glycoprotein (Pgp) or the constitutive expression of multidrug resistance-related proteins (MRPs) that are homologous to Pgp, and like Pgp are members of the ABC family. They transport a multitude of drugs outside of the cell and are not specific.

The most recent strategy in cancer treatment is the concomitant administration of antitumoral drugs with a MDR-modulator, which can cause tumor cell death. Therefore, many efforts are geared towards the synthesis and the study of MDR-modulators that specifically inhibit the transporter and allow the antitumor drug to stay inside the cell and provoke cell death.

Lee *et al*. [5] identified a series of dihydropyrroloquinolines derivatives that reverse Pgp-mediated MDR without antagonizing MRP. Among these derivatives, PGP-4008 was the most promising. PGP-4008 activity was characterized with different cell lines, and the authors showed that it potentiates the cytotoxicity of Pgp substrates only on cell lines that overexpress Pgp, and increases drug accumulation inside the cell. PGP-4008 has no effect on non-Pgp-overexpressing cells, nor does it affect the toxicity of non-Pgp substrates. PGP-4008 is specific to Pgp and does not antagonize MRP. *In vivo*, tumor growth was significantly slower when mice were treated with a combination of PGP-4008 and doxorubicin. Although more clinical studies are necessary to better characterize PGP-4008, it seems to be a promising drug because it specifically reverts Pgp-mediate MDR.

5 Lee, B.D. *et al*. (2004) Synthesis and evaluation of dihydropyrroloquinolines that selectively antagonize p-glycoprotein. *J. Med. Chem*. 47, 1413–1422

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Business

Collaboration

Partnership for cutting-edge genomics research

Children's Memorial Institute for Education and Research (http://www.childrensmemorial.org; CMIER, http://www.cmier.org) and The Translational Genomics Research Institute (TGen; http://www.tgen.org) have announced a partnership to conduct genomic research into childhood illnesses and help better define their relationship to adult disease.

Mary J.C. Hendrix, President and Scientific Director of the Chicago-based CMIER and Professor of Paediatrics at Northwestern University's Feinberg School of Medicine, said: 'This partnership will enable us to build a world-class genomics program that will profoundly impact human health and accelerate the rate of discovery into the molecular components of childhood diseases.'

The two institutes will conduct research on a broad spectrum of problems, including brain disorders such as schizophrenia, behavioural disorders, multiple sclerosis, cancer and autoimmune diseases. The research will focus on detecting genetic markers, using the latest DNA microarray technology, and finding faster ways of moving discoveries from the laboratory into the clinical setting.

TGen's President and Scientific Director, Jeffrey Trent, commented: 'Our collaboration with Children's Memorial further strengthens TGen's mission to advance research in an expedited manner. The sequence of the human genome has fuelled a rapid increase in gene discovery and analysis and our work with Children's Memorial will hopefully answer a number of questions surrounding childhood disease.'

Business was written by
Joanne Clough

People

Appointments

Dynavax names VP and Chief Business Officer

Dynavax Technologies (http://www.dynavax.com) has announced the appointment of D. Kevin Kwok as Vice President and Chief Business Officer.

Kwok was most recently VP for the transaction advisory group Clearview Projects, where he was responsible for the start-up and client management of the San Francisco practice. He brings more than 18 years worth of diverse industry experience with both pharmaceutical and biotech companies in various commercial areas.

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