Box 1. Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive inherited disorder that causes an abnormality in airway epithelia that prevents those affected clearing bacteria from their lungs and leaves them vulnerable to serious infections. The mutation, which is common and is carried by 1 in 22 Caucasians, causes a defect in the CF transmembrane conductance regulator (CFTR) involved in chloride ion excretion from airway epithelia. The defect closes the channel and prevents chloride ion excretion. This leads to a dramatic reduction in excretion of sodium ions and water. With little water in the airways, the mucus becomes thick and sticky and susceptible to colonization by *Pseudomonas aeruginosa*.

Current therapies for CF include physiotherapy to clear the airways, antibiotics to clear lung infections, treatments to improve sodium and chloride ion transport and reduce mucus viscosity, DNAse therapy to degrade the build up of DNA from dead inflammatory cells in the airways, and gene therapy to rectify the defective CFTR gene. The use of treatments has considerably expanded the lifespan of people with CF, but none of them is wholly effective.

to help them identify gene function. Of those used for comparison, *Escherichia coli* was the nearest relative, a fortunate discovery as a great deal is already known about the *E. coli* genome.

This technique enabled the researchers to predict the function of 54% of the *P. aeruginosa* genes. Apart from the expected array of 'housekeeping' genes required for motility, adhesion, DNA replication, protein synthesis, cell-wall biosynthesis, and biosynthesis of amino acids, nucleic acids and co-factors, the genome contains a surprisingly high proportion of genes that regulate transcription (341 genes) and genes that encode molecular transporters (408 genes).

The size and diversity of the *P. aeruginosa* genome, along with the high number of regulatory genes that enable the bacterium to switch genes on and off as the prevailing conditions dictate, helps explain the pathogen's ability to survive in many different conditions. Its remarkable ability to resist attack by antibiotics is also easy to explain now the genome has been sequenced: '*Pseudomonas aeruginosa* has many genes that allow the bacteria the capacity for exceptional adaptability. We now also know that it has quite a few pumps to extrude antibiotics before their intracellular concentrations reach a crucial threshold', says Christopher Penland, CFF Director of Research.

Availability of gene chips

The *P. aeruginosa* genome sequencing project was a prime example of the benefits of cooperation between scientists. In all, 61 *P. aeruginosa* experts were involved in interpreting the genome sequence alongside the central research team. The CFF plans to encourage further cooperation by making *P. aeruginosa* gene chips available at a discounted price to CF researchers. 'The gene chips are projected to be available in late 2000 or early 2001', says Penland.

In return for the cut-price gene chips, researchers will be expected to submit their data to a common database. 'Once data are submitted, researchers can examine data on an exclusive basis for 6 months. After the 6-month period has expired, the data will be accessible to all individuals who have submitted data', explains Penland. Researchers will also have access to a data mining utility software program known as Genomax that is licensed by the CFF from Informax, and that data will be available to be examined via the Internet 24 hours a day, 7 days a week.

Reference

1 Stover, C.K. *et al.* (2000) Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature* 406, 959–964

Higher-throughput automated systems for ion-channel screening

Kathryn Senior, freelance writer

Screening for drugs that target ion channels is currently difficult and slow. Recently, an Australian company based in the USA announced further testing of a prototype system they have developed to enable higher-throughput screening of compounds that bind to a whole spectrum of ion-channel proteins. Andy Blatz, Director of Cell-Based Screening Technology at Axon Instruments (Foster City, CA, USA) says that the prototype system will require 9–12 months of testing before the device is launched, probably next November.

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Problems with current ion-channel screening methods

Ion channels are found in virtually all cell types, playing a vital role in the CNS, contributing to immune cell function, and being responsible for much of the transport that occurs in the kidney, gut and lung. Traditionally, screening for compounds that affect ion-channel function has been slow, laborious, relatively inaccurate, and has depended on indirect methods such as fluorescence systems.

'The real Achilles heel of fluorescence measuring systems is that they have no control over the membrane potential of the cell. This is a major problem in the case of voltage-dependent ion channels,' points out David Owen, Managing Director of the Channelwork group at CeNeS Pharmaceuticals (Cambridge, UK). CeNeS is also developing an automated HTS system for ion-channel drugs (see Box 1). In many cases, says Owen, these channels exhibit state-dependent modulation by drugs that might not be detected at all using fluorescence systems.

Conventional electrophysiology techniques such as oocyte voltage- or patch-clamping also have their limitations. 'These typically monitor only one cell at a time and measure electrical activity of ion channels indirectly,' says Chris Mathes, Manager of High-Throughput Electrophysiology Screening at Axon.

As electrophysiological screening techniques have been so limited, a large number of lead compounds have turned out to be false-positives or, worse still, false-negatives: 'we could be ignoring potential blockbuster drugs,' says Blatz. To pick up all compounds that affect ion channels, researchers need to be able to measure every tiny fluctuation in the current passing through an ion channel. 'Conventional systems do not allow close enough scrutiny of ion channels to enable such subtle changes to be detected,' he adds.

Automating the screen

The new Axon system uses voltage clamping as its basis, enabling the direct measurement of ion-channel currents in oocytes that express large quantities of specific ion-channel proteins. 'This unique expression system enables the measurement of electrical activity of a single type of ion channel *in situ* in a biomembrane, and the oocyte does all the work,' says Blatz.

The ion-channel-expressing oocytes are then placed in a specially developed chamber and localized in 3-D space. Currently, Axon's cassette holds eight such chambers that are positioned on a probe station that carries eight sets of double electrodes. These electrodes move down automatically to the oocytes by a synchronized alignment system. 'This represents a great advantage over conventional oocyte screening technology in which a highly skilled operator aligns electrodes and guides them towards the oocyte membrane using micromanipulators and a microscope,' says Mathes. The computer-controlled automated system maintains solution flow and applies the test drug at a set concentration for a set time. Experiments typically include a period of control, followed by application of the test compound, followed by more control fluid. The measurements of electrical activity across the ion channels between the control and test phases gives a measure of percentage inhibition.

Is this true high-throughput technology?

'The Axon automated oocyte system will speed-up the process of screening voltage-clamped cloned ion channels and should make it possible to use such techniques effectively in drug discovery programmes that target ion channels,' concludes Owen. However, he cautions that the method is likely to find utility in secondary and tertiary screening in which cloned ion channels are the target, rather than in true high-throughput primary screening. Whilst Mathes and Blatz agree that this is true at the moment, they stress that the more that systems can be operated in parallel, the greater the throughput. 'It will soon be possible to screen 48 oocytes at the same time but, even within the next five years, it should be possible to develop an automated patch clamp system in which 1000 mammalian cells can be tested simultaneously,' predicts Blatz.

The latest phase of testing is in progress in association with Roche Bioscience (Palo Alto, CA, USA), which is currently advising on feature specification of the final prototype. Roche is testing the system using sodium, potassium and calcium channels, a variety of neurotransmitter-activated channels and G-protein-coupled ion channels, and this is expected to be completed by autumn of next year. Ian Massey, Senior Vice-President of Neurobiology at Roche said that, 'While we routinely examine the effects of drugs on receptors and ion channels, electrophysiological studies currently provide only a secondary or tertiary screen for compounds. Axon's automated oocyte system could improve our throughput significantly and will

Box 1. The Channelwork patch-clamp system

The Channelwork division of CeNeS plc has also been addressing the need for improved HTS systems for drugs that target ion channels. 'Channelwork has developed a fully-automated patch-clamp system that uses mammalian cells and cell lines', says Owen. Current AutoPatch™ prototypes are 'single-click' systems requiring no experience of electrophysiology, are highly compact and can be bench-mounted. Several systems can be controlled by a single operator enabling several experiments to be performed in parallel. This system is currently being further miniaturized and scaled-up to produce a parallel patch-clamping platform that will enable ≈50,000 data points a week to be collected from patch-clamped cells. 'The system is likely to be complementary to the Axon automated oocyte system, which could be used to address different questions and channel types downstream of an AutoPatch primary screen', concludes Owen.

also enable us to combine the sensitivity of electrophysiology with much greater capacity.'

The future

The savings that automated screening systems could bring in terms of time and the reduced need for skilled operators could, hopes Blatz, provide new impetus to research. 'If highly skilled neurophysiologists are freed from micromanipulation and single-cell experiments, they will have much more time for innovative thinking and planning,' he says. In the long-term, Blatz and Mathes also envisage that Axon might be able to couple its genomic and proteomic developments with HTS technology. 'We cannot predict the outcome of this, as technology will need to advance to even make it possible, but this is an area that we do not want to miss out on,' concludes Blatz.

HTS in the new millennium

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Screening, in particular high-throughput screening (HTS), has taken on a broader role within discovery science as increasingly automated and robust technologies have been brought online. Consequently, the scope of the Society of Biomolecular Screening (SBS) Sixth Annual Conference and Exhibition entitled *Screening in the New Millennium* (6–9 September 2000 in Vancouver, BC, Canada) was considerably broader than previous SBS meetings, and was markedly less focused on techniques and equipment. Successful application of the principles of HTS, both for lead identification and optimization, were presented. In addition, the connection between molecular screening and compound synthesis to reduce the time taken to achieve discovery milestones was strengthened. Other presentations covered new technologies, particularly the use of microscopy in HTS, which is now more fully developed to the point of practical application in the HTS laboratory.

New leads

As the membership of the SBS has grown in number from a few hundred to a few thousand over the past six years, the operating principles of the HTS laboratory have become less idiosyncratic. This is becoming manifest in the number of new leads that are emerging as a direct consequence of the HTS laboratory. The realization of this promise was substantiated by presentations from numerous companies in several target classes. Highlights included:

- The identification of potent, selective inhibitors of the human inducible nitric oxide synthase (iNOS), discovered at Pharmacopeia and being developed by Berlex Biosciences (Kirk McMillan; Princeton, NJ, USA)
- The identification and optimization of novel non-peptide chemokine receptor 1 (CCR1) antagonists, also at Berlex Biosciences (Meina Liang; Richmond, CA, USA)
- The identification and optimization of novel melanocortin MC_1-receptor agonists at Trega Biosciences (Timothy Gahman; San Diego, CA, USA).

Stephen Rees (GlaxoWellcome, Stevenage, UK), and Matthew Sills (Novartis, Summit, NJ, USA) noted important interactions between the choice of assay technology and outcomes of screening. Sills suggested that for certain target types, the use of at least two different assay technologies per target would be justified to avoid false-negatives in screening.

Combinatorial chemistry and HTS

An increasing coordination of the efforts of combinatorial chemistry groups with those of the HTS laboratory was also noted. HTS laboratories have always needed close association with compound storage and retrieval. By more tightly linking screening with *de novo* synthesis, particularly in the context of lead generation through robotic and combinatorial chemistry, increases in efficiency are anticipated. The debate between using large combinatorial libraries (advocated by Maria Webb of Pharmacopeia, Princeton, NJ, USA) and iterative robotic synthesis (advocated by Peter Myers, DuPont Pharmaceuticals, San Diego, CA, USA) was discussed in terms of past successes, with the primary difference between sequential iterations and parallel screening being the time to achieve a lead.

Large combinatorial libraries provided optimizable leads in a variety of programs, highlighted by the successful identification and optimization of receptor antagonists specific for the human bradykinin B_1 receptor at Pharmacopeia, while iterative synthesis is being increasingly viewed as an aid to lead optimization (Raju Mohan, Berlex Biosciences, Richmond, CA, USA). Approaches towards

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