for *in vitro/in vivo* correlations at the level of intestinal permeability prescreens, such as the Caco-2 intestinal epithelial cell system, and also drug interaction and metabolism prescreens using liver microsomes. Upon obtaining *in vitro* human half-life prediction data, which provide information on bioavailability limitation factors, lead compounds are then tested *in vivo*. Thus, *in vivo* study is minimized while, at the same time, vital early-stage information is provided to formulators.

The hot topic of gene chips was addressed by Dr David Bailey (Incyte, Palo Alto, CA, USA) and Dr Gordon Smith-Baxter (Pharmagene, Oxford, UK). In theory, chip technology allows the simultaneous display of expression data from thousands of genes and has a resolution that can detect single base polymorphisms. Ideally, the large genomic databases that are available allow companies to target specific gene families, followed by application to human isolated tissue for functional assay and screen of potential discovery compounds. Many participants did not yet trust the gene expression end point, given that some data showed a nonlinear relationship between protein expression and RNA production. Beyond the hype, the technology is not yet at the stage where the chips can give quantifiable data that relate to real biological consequences. Also, multiple gene diseases may not be adaptable to this technology given the role of environmental factors. Nonetheless, this exciting technology has potential as part of early-stage discovery programmes, perhaps more immediately to screen ligand binding for single gene conditions in animal models.

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Society for Biomolecular Screening – highlights

The *3rd Annual Conference of the Society for Biomolecular Screening* (SBS) held in San Diego, CA, USA attracted 546 registrants and 180 exhibitors (77 exhibits). The increased attendance over last year's meeting demonstrates the greater emphasis placed on 'screening' in the pharmaceutical and biotechnology industries. At the annual conference of this society, short, but highly informative, courses were held prior to the proceedings (e.g. 'Genomics in Drug Discovery'), and tutorials were given by the manufacturers of screening equipment and reagents. Moreover, these technology-oriented meetings are useful for learning about existing methods and for introducing new instrumentation.

Assay platforms

This first session included talks ranging from functional cell-based assays to screens based on affinity selection, fluorescence polarization (FP) and electrochemical detection. Dr Daniel Gil (Allergan, Irvine, CA, USA) described the advantages of functional high-throughput screening (HTS) using receptor selection and amplification assay technology (RSAT). In this method, mixed populations of cells expressing different receptors are engineered to proliferate in response to ligands. Screening for α-adrenoceptor subtype selective compounds was also described using RSAT. For most receptor screens, the particular pathway used in a given therapeutic target is not known, and the functional screen provides a variety of conformational possibilities. Receptors signal through multiple biochemical pathways, and the conformation of the receptor varies depending upon the pathway selected. Although a compound must bind to the receptor to be active, it was noted that binding assays might actually be misleading because the activity in functional assays may be less than in binding assays. The activity of ligands selected from the functional assay was shown to correlate with the therapeutic effect in cornea reflex studies. Partial agonists could also be identified by RSAT.

Dr Dennis Church (Glaxo Wellcome Research, Geneva, Switzerland) discussed cell-based functional assays as alternatives to electrophysiological methods for measuring activation of the purinergic receptors P2X, P2X2/3 and P2X4. These receptors are a family of ATP-gated channels that are permeable to both Ca^{2+} and Na^{+}. HEK cells expressing P2X7 receptors are made permeable by the activation of P2X7 receptors. The increased permeability was measured using Yo-Pro-1 iodide; this complexes with DNA, and the fluorescence produced can be measured using a cytofluorometer and a fluorescence imaging plate reader (FLIPR). The calcium-sensitive indicator, Fluo-3, and the membrane-potential-sensitive dye, DIBAC, were used for measuring P2X2/3 and P2X4 activation, respectively. These methods allowed a throughput of 2,000 samples per day.

The Raf kinase pathway has several component kinases, each of which is a good drug target for oncology. Dr Brad McDonald (Glaxo Wellcome, Research Triangle, NC, USA) described the use of a single scintillation proximity assay (SPA) to identify inhibitors of Raf/MEK/ERK. This screen requires His-tagged c-Raf-1 to be expressed in the activated form and the other kinases expressed, in an inactive form, as glutathione-*S*-transferase (GST) fusion proteins in *Escherichia coli*. Phosphorylation of a biotinylated peptide substrate by ERK-2 was detected by binding to scintillant beads.

Dr John Wang (Chiron, San Francisco, CA, USA) described mini-spin affinity columns and mass spectrometry to deconvolute and identify structures of hits from pools of compounds. The mini-columns can be parallel-processed and a throughput of 200 samples per day was described.

The use of electrochemistry as a real-time method for measuring inhibitors

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of protein–protein interaction was described by Dr Holden Thorp (Novalon Pharmaceutical, Chapel Hill, NC, USA). In this method, surrogate ligand labelled with horseradish peroxidase (HRP) binds to GST–SrcSH3 fusion proteins immobilized in a redox hydrogel [PEGDGE and poly(vinyl imidazole) labelled with Os(bpy)2Cl^{+}] that is attached to an electrode surface. Hydrogen peroxide is released by HRP turnover, and the resulting electrons are detected. The surrogate ligand can be displaced by a small molecule ligand, in this case peptides displayed by phage display, resulting in decreased current. This method requires optimization and, as presented, microgram amounts of protein.

Dr Ramakrishna Seethala (Bristol-Myers Squibb, Princeton, NJ, USA) described FP as a simple, homogeneous system that is adaptable for any type of screen in which the fluorescently labelled partner increases or decreases in size. The lck protein tyrosine kinase assay was used as an example in which the peptide phosphorylated by lck was trapped with an antiphosphotyrosine antibody, resulting in an increased polarization signal. In addition to this standard assay, an FP competitive assay was used to circumvent the use of large amounts of pY antibody. In this method, the tyrosine phosphorylated peptide competes with fluorescent phosphopeptide bound to pY antibody.

Toxicology and drug metabolism

Dr Ronald Borchardt (University of Kansas, KS, USA) described the use of Caco-2 cells in drug discovery. Early identification of critical pharmaceutical qualities of compounds, such as intestinal permeability, was possible by using Caco-2 cells in HTS. *In vitro* drug metabolism studies using Caco-2 cells, hepatocytes and HPLC for HTS studies were described by investigators from Merck and Pfizer. A new cell line derived by expressing high levels of cytochrome P450 in Caco-2 cells was used for combining intestinal permeability studies with drug metabolism in the liver (Dr Charles Crespi, GENTEST, Woburn, MA, USA). These *in vitro* methods could be better than investigating the compounds in animals because the pharmacokinetics of drugs in rodents differs significantly from those in humans. The throughput of efficacy studies in rodents could be increased by testing multiple compounds simultaneously (J. Schaffer, Glaxo Wellcome, Research Triangle, NC, USA).

Diversity analysis

This session focused on the idea that large random libraries are not very useful for primary screening, and the move was towards making smaller, more focused libraries for lead optimization. Dr Martin Haslanger (Sphinx Pharmaceuticals, NC, USA), the plenary speaker for this session, emphasized the derivation of new medical targets from genomic information and their use in high-efficiency screens. Combinatorial chemistry could be used to fill-out the molecules needed for SAR studies. The selection of compounds must be based on molecular diversity and the therapeutically useful chemical space. It is believed that deriving the therapeutically useful target space for each class of target will drive synthesis and chemical diversity using combinatorial chemistry. The concept of virtual libraries to generate neighbourhood space was presented by Dr Allan Ferguson (Tripos, St Louis, MO, USA). Actual synthesis is product-driven instead of reagent-driven. Dr Ron Barrett (Affymax Research Institute, Palo Alto, CA, USA) believes that large combinatorial libraries are beneficial because small differences in chemical structure can make large differences in activity. About two dozen diverse scaffolds are used for the large unbiased libraries. These libraries are made on encoded beads, and the compounds are released by photolysis or by acid cleavage for screening. Affymax has designed screening technologies to handle one million compounds per day. Dr Steve Teig (CombiChem, San Diego, CA, USA) described three types of libraries. Diversity libraries are those that are low in information content and generate few hits in screening. Coverage libraries are also low in information content but generate greater numbers of screening hits. Informer libraries are the best, with high information content and the capacity to generate many hits in a screen. Teig believed that a 10,000 member informer library was sufficient for screening.

Future outlook

Dr Johan Roeraade (Royal Institute of Technology, Stockholm, Sweden) presented a plenary lecture on performing nanoscale chemistry. Deep reactive ion etching and laser-assisted machining has made it possible to construct microvessels and flow systems on the nanometre scale with precision. Silicon wafers etched with 500,000 vials were shown. Micropipettes and piezoelectric-driven inkjet-like dispensers were used for distributing reagents using robots equipped with interactive video, which allowed continuous monitoring of the microchip. Liquid-lock and continuous addition of solvent were used to avoid evaporation. The microvial technology could be developed into a generic platform for nanoscale chemistry on which a vast number of solid phase reactions could be performed. Detection was by fluorescence and MALDI-TOF-MS.

Dr Randy Scott (Incyte Pharmaceuticals, Palo Alto, CA, USA) gave the plenary lecture on genomics. The acceleration in the rate of DNA sequencing and the ability to store, retrieve and analyse information from the genomics effort are directly related to the improvements in the computer industry. It correlates well with Moore's law, which states that computer memory will double every year while costs are cut by half. Incyte sequences about 15,000 clones a day, over 600 Mb of DNA per year, and sequencing will exceed 100,000 megabases per year by the year 2,000, providing over 30-fold coverage of the human genome at that time. About 2.26 million sequences are currently available at Incyte. The creation of expressed-sequence tag libraries has been a powerful tool for the genomic revolution. Effort is under way to sequence and map the 100,000 human genes in order to develop annotated databases for sequence homology, structure, expression patterns, function, polymorphism, and so on, and to use the

genomic databases to answer biological questions. Unlike the current approaches, these databases will allow a systematic information-based approach to studying the biology of thousands of genes at a time. High-density microarray technology developed by Affymetrix is being used to put every gene on a chip. These chips can be probed to determine when a gene is turned on or off in a variety of cells or tissues of choice and the effect of drug treatment on the expression of the gene. For example, all 20,000 genes expressed in activated T cells can be put on a chip and probed for effects by various immunosuppressive agents or ligands. Comparative genomics is also being performed at Incyte. Relational databases using information from the microarrays are being developed for building future bioinformatic capabilities at Incyte.

Dr Robert Jackson (Chiroscience, Cambridge, UK) was the plenary speaker at the 'Bioinformatics' session. Biological systems are complex but can be modelled using multiple parameters. Bioinformatics has been driven by the genomics effort as well as by HTS. The database will allow prediction of drug metabolism and toxicity, as well as the response duration from pharmacological information. At Agouron, these databases were used for the development of their HIV protease inhibitor. Information relative to the life cycle of the virus, the pharmacokinetic parameters of the protease inhibitor and the activity of the compound against the virus were taken into consideration when building the database.

Dr Kevin Oldenburg (Dupont Merck, Wilmington, DE, USA) spoke about his work in miniaturization of HTS to increase screen throughput. He presented a 9,600-well format for soluble enzyme, microbial-based screens, a 2,400-well format (0.2 µl per well) for enzyme and cell-based format (0.5 µl per well) and 960 wells for SPA receptor assays (20 µl per well). The liquid is dispensed 'on the fly' by careful timing for 10 nl delivery. The wells were designed with slanted sides to be able to receive compounds on beads. Evaporation results in a 28% loss of volume within 15 min, and humidity control is necessary. Microbial assays used the green-fluorescent-protein reporter. A matrix metalloproteinase assay for MMP-3 was described using fluorogenic reporters that had sufficient sensitivity for reading in the 9,600-well format. The equipment was produced in collaboration with BioDot in Canada.

In addition to the sessions mentioned above, there were 70 poster presentations. The adaptation of new technology, such as time-resolved FP to screening, and SPA to develop homogeneous screens, was described in these posters. Also, 72 companies exhibited their technologies to the registrants of the SBS.

All in all, this Annual Meeting was very successful; new information was exchanged between friends, and new friends were made.

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Electrical genes

Japanese chemists have developed an electrochemical sensing system for genes. The technology offers the possibility of replacing expensive, short-lived and troublesome radioisotopes used in medical labelling with an inexpensive and safer approach and could speed up drug research into genetic diseases.

Toshihiro Ihara and his colleagues in the Dept of Chemical Science and Technology at Kyushu University, Japan describe in a recent issue of *Chem. Commun.* (1997, 1609–1610) how they have modified an electrode sensor to respond to the presence of specific genes without the need for a radioisotopic label. They constructed their gene-sensing system using an active iron molecule, a so-called ferrocene, and a modified DNA probe with a nucleotide strand anchored to a gold electrode. The ferrocene undergoes reduction–oxidation reactions, depending on its chemical surroundings, and produces an electrical signal as it does so. The nucleotide strands – short lengths of DNA, chosen to target a known gene – produce a response in the presence of the target only.

'The detection procedure is very simple,' explains Ihara. The electrode sensor is placed in a sample solution containing the DNA of interest, such as a body fluid sample from a patient suspected of having a genetic disease. If there is a match between the nucleotide strand and a gene in the sample then an electrical signal will flow in the electrode and be easily detected.

By carefully choosing the nucleotide strand to match disease-inducing genes, the investigators can effectively detect the presence of such a gene in a patient without exposing the patient to radioisotopes. 'The strategy of the sensor is universal,' says Ihara. 'In principle, all genes responsible for certain diseases can be the target of the sensor by choosing an appropriate sequence of the redox active probe (ferrocene-modified DNA probe).'

'Radioisotopes are widely used as labels because of their high sensitivity,' explains Ihara. 'However, because of their hazardous nature and short shelf-life, the development of an alternative detection system is eagerly hoped for.' His team's system could be just such a convenient and practical alternative.

The researchers concede that they have not yet optimized the measurement conditions of the device, but they are working on it. 'We have now confirmed that the sensor responds to at least attomole concentrations (10^{-18}); this is of course sufficient for practical use,' adds Ihara. Industrial collaborators will hopefully commercialize the system. 'Our interest is in the performance of the system for detecting disease-inducing genes. Commercial development is beyond our scope, although of course we hope for it,' Ihara remarks.

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