Instead of trying to engineer stable PKSs, these workers have decided to use combinatorial biosynthesis techniques to recombine intact modules from what Khosla calls the 'vast natural repertoire of PKSs', which can then be used to create the next generation of PKSs. Furthermore, the enzymatic repertoire, and therefore the chemicals produced by these PKSs, could be extended further by 'interweaving' PKS modules with natural non-ribosomal peptide synthetases.

One problem with this approach is that all the current evidence suggests that natural modules normally only select their natural counterparts. Therefore, assembling artificial complexes will require some method of cross-talking during the combinatorial biosynthesis of modules that might not normally assemble, to prevent the same problems of instability of the early synthetic PKSs.

Khosla and his team have now devised a method to overcome this problem, leading to the production of stable PKSs[1]. They have studied four individually expressed, catalytically active modules from the erythromycin PKS, and found that different combinations of the individual modules can extend a given diketide substrate into the corresponding triketide. These combinations have been found to have similar reaction kinetics implying that the same mechanism is responsible for each reaction. Furthermore, they have noted that short amino acid linkers between modules play a crucial role in the assembly of modules into functional structures. They now believe that by the appropriate engineering of these linkers, they can make modules work together, that normally would not do so.

In the past few years, Khosla and other workers have produced hundreds of new polyketides. Khosla has highlighted that possibly the most interesting compound produced by genetic engineering of PKSs is the ketolide, which was produced by Robert McDaniel and colleagues (Kosan Biosciences Inc.), and which simplifies access to a new class of anti-infectives currently derived semisynthetically from erythromycin via 10–15 chemical steps[2]. The development of linker technology will allow researchers to extend the approach still further to generate greater molecular diversity. Khosla predicts that the first genetically engineered polyketides may enter clinical trials within the next two to four years.

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David Bradley
tel/fax: +44 1954 202218
web: http://www.camsoft.com/elemental/

Molecular labels, signalling and detection

Various types of molecular labels and cell signals are now used for DNA detection. Because DNA does not have intrinsic properties that are useful for direct high-sensitivity detection, many DNA detection assays require a label (that is a secondary detection technology). Biosensors are also assuming importance for *in vivo* diagnostics. The current emphasis is on enhancing sensitivity, accuracy and speed of all these methods and these topics were discussed at the third annual Cambridge Healthtech conference entitled *Molecular Labels, Signalling and Detection* held in San Diego (CA, USA), 12–13 April 1999. The conference covered important microchip-based DNA detection methods, opticochemical sensor technologies and novel physiological probes for living cells.

Microchip-based detection of DNA and proteins

Electrochemical detection of DNA was described by Holden Thorp (University of North Carolina, Chapel Hill, NC, USA). The principle of this method is the use of a mediator to transfer electrons from DNA to a miniaturized electrode, where DNA can be immobilized for maximum specificity and can then be detected by cyclic voltametry. Metal oxide electrodes modified with phosphonate-tethered oligonucleotides offer concentration-dependent detection of unlabelled DNA at surface densities approaching one femtomole per square centimetre. With inosine-substituted probes, guanine gives rise to hypoxanthine (this is the source of the name Xanthon (Research Triangle Park, NC, USA) a company that is commercializing this technology).

Jon Kayyem (MicroSensors Inc., Pasadena, CA, USA) described the development of a hand-held microchip reader for biodetection of DNA. This reader contains several electronically active microelectrodes with specific DNA-capture probes, and these are linked to the electrodes through 'molecular wires'[1]. Target DNA or RNA is labelled with ferrocene, a redox label[2] and signals are generated by probe–target

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interaction (i.e. hybridization). Potential applications for this technology include diagnosis of infectious diseases, blood screening, pharmacogenomics, drug screening and disease management, with advantages including simplicity and low cost.

Novel tools for creating and reading microarrays were described by Stanley Rose (Genetic Microsystems Inc., Woburn, MA, USA). The array-concept is based on fluid transport controlled by using surface tension forces. These technologies are still expensive and their limitations include a lack of standard procedures, unproven reliability of instrumentation for deposition of array elements, and limited access to chemistries for making arrays as these are often proprietary. Integrated instruments such as GMS 417 and GMS 418 (Genetic Microsystems), however, have been developed which utilize a spotting technology and other proprietary design features, such as fast scanning and high sensitivity, for successful function. Both of these instruments are available at affordable prices, can use conventional chemistries and can be used for applications such as gene expression monitoring, mapping clones, mutation detection and drug discovery[3].

Enrique Dalmasso (Ciphergen Biosystems, Palo Alto, CA, USA) described the ProteinChip™ arrays which integrate several functions, including on-chip parallel detection, purification, quantification, structure, and functional analysis of femtomole-quantities of proteins taken directly from their native environments. ProteinChips are read by a surface-enhanced laser desorption/ionization (SELDI)-based system at a rate of 10 s^{-1} and identifies the retained proteins on the basis of their molecular weights. Receptor–ligand screening, epitome mapping and protein–protein, protein–DNA and protein–drug interaction studies can be performed directly on the chip. Other applications of the technology include gene expression monitoring, differential protein display and characterization of signal transduction pathways. This technology enables phenomics (the phenotype-based approach to the characterization of disease markers and drug target proteins), which is analogous to genomics (the gene-based approach to the same goals).

Novel methods of DNA detection

Information on dendritic DNA (3DNA™) nucleic acid amplification was presented by Thor Nilsen (Genisphere Inc., Bala Cynwyd, PA, USA). Dendritic molecules are highly branched arborescent (tree-shaped) structures that can be extremely useful for the development of nucleic acid diagnostics as signal amplification tools[4]. Furthermore, because of the relatively large size of nucleic acid molecules, 3DNA can be readily labelled with numerous fluorescent compounds. Four-layer 3DNA molecules labelled with Cy3™ and fluorescein isothiocyanate (FITC) are called DNA dymons and can enhance nucleic acid detection by 50–100-fold. DNA dymons also enable the detection of single-copy oligosequences from nanogram-quantities of human DNA using Southern blot assays. Specificity to various DNA sequences is conferred to the dendrimers by hybridizing and covalently cross-linking oligonucleotides to the single-stranded surface of the dendrimers.

Another technique for DNA detection is the use of molecular beacons, which are oligonucleotide probes that become fluorescent upon hybridization (Janet Moore, Stratagene Inc., La Jolla, CA, USA). Sentinel™ molecular beacons (Stratagene) are multiple systems that exploit the advantages of this technology and can be used to monitor polymer chain reaction (PCR)-product formation during or after the amplification process. Their use eliminates the need for gel analysis following thermal cycling, therefore saving time and decreasing the potential for contamination of laboratory equipment or reagents. The probes confer a high specificity of target detection and have an ability to detect single nucleotide mismatches. Applications for this technology include quantitative analysis of genomic DNA, gene expression analysis and diagnosis of infections. Allelic discrimination kits have been designed to detect various mutations. One example of this is the MTHFR (methylenetetrahydrofolate reductase, a key enzyme in homocysteine metabolism) gene, mutation (C to T substitution, Ala→Val) of which has been linked to an increased risk of cardiovascular disease and neural tube defects[5].

Robert Kwiatkowski (Third Wave Technologies, Madison, WI, USA) presented information on direct detection of nucleic acids using the Invader™ (Third Wave Technologies) assay. This test uses structure-specific endonucleases, or Cleavase® enzymes, which form the basis of the company's CFLP® (Cleavase Fragment Length Polymorphism) assay, and is dependent on the ability of the Cleavase enzyme to recognize secondary DNA structures that form as DNA cools following brief thermal denaturation[6]. Cleavase enzymes cut these structures, creating a unique 'fingerprint' pattern of bands. Invader and signal probes are designed to hybridize overlapping sites on the target strand such that the Invader probe displaces a part of the signal probe. This process forms a structure that a Cleavase enzyme will recognize and cut, creating detectable products. The Invader assay can be used both to discriminate single nucleotide polymorphisms (SNPs) directly from genomic DNA, and for direct expression analysis using mRNA. Amplification with this assay is 60,000-fold and it yields significantly better specificity and discrimination than hybridization-based methods. Potential applications are in the detection of minimal residual disease in cancer patients and HIV, where it can detect less than 50 organisms in a sample.

Opticochemical sensor technologies

William Velander (Virginia Polytechnic Institute, Blackburg, VA, USA) described a Long Period Grating (LPG) fibre optic sensor, which can be used to detect small changes in index of refraction within the surrounding field. The device, Lunascan™, is simple in construction and also has chemosensing abilities that enable the detection of biochemicals. The NanoOptoChemical devices which include PEBBLES (Probes Encapsulated By Biologically Localized Embedding)-sensors, were described by Martin Philbert (University of Michigan, Ann Arbor, MI, USA)[7]. These are wireless and fibreless sensors, with radii as small as 10 nm, and can be inserted into cells using a biolistic (ballistic microprojectile) device such as a gene gun (Bio-Rad Inc., Hercules, CA, USA) for real-time *in vivo* chemical mapping of sub-cellular pathological processes such as cancer.

Steven Barnard (Illumina Inc, San Diego, CA, USA) described the proprietary technology which combines the ability to microfabricate optically addressable arrays of micrometre-size wells and optically encoded libraries of microspheres that contain sensing chemistry. Every probe on each array can be checked in parallel and have a unique code that distinguishes it from all other probes. These arrays can be used for SNP genotyping with a projected capacity of 3,000,000 SNP genotypes per day.

Light- and fluorescence-based physiological probes in living cells

Shumin Nie of Indiana University (Bloomington, IN, USA) described the use of luminescent quantum-dot bioconjugates for *in vivo* sensing. Quantum dots are metal and semiconductor particles on the nanometer scale that have unique optical, electronic and structural properties that are not available on isolated molecules. Large biomolecules are covalently linked to the solubilized quantum dots and the bioconjugates are found to be suitable for use in cell biology and immunoassays[8]. Other uses include multiplex gene expression assays and quantum-dot-based molecular beacons. A potential clinical application is in the detection of viral RNA, in which as few as 100 copies of hepatitis and HIV viral RNA per millilitre of blood can be detected.

Harry Crissman (Los Alamos National Laboratory, Los Alamos, NM, USA) demonstrated the feasibility of monitoring the binding of fluorescent chemotherapeutic drugs in living cells and correlated drug uptake to cell size and cell-cycle position. Flow cytometry (FCM) is used for the measurement of individual cells, chromosomes, particles and molecules[9]. These novel assays will expand the applications of quantitative studies on the binding of various chemical agents to DNA and other molecular targets in cells. This technology has the potential to be used in the design and testing of new fluorescent drugs as well as for the screening of environmentally toxic compounds. John Steinkamp, also at Los Alamos National Laboratory, described phase-sensitive FCM and fluorescent lifetime-based sensing technology for analyzing cells labelled with fluorescent probes. By modulating the intensity of the laser excitation source at high frequency and using phase sensitive detection methods, the excited-state lifetimes of fluorescent markers are measured as cells pass across the laser beam. The measurement of fluorescence lifetime can be used as a spectroscopic probe to study the interaction of fluorescent markers with their cellular targets.

Methods for monitoring *in vivo* gene expression by magnetic resonance imaging (MRI) were presented by Thomas Meade (Beckman Institute, California Institute of Technology, Pasadena, CA, USA). Meade and coworkers have tested several new 'smart' MRI contrast agents that influence the nearby water protons in a conditional manner. This modulation is triggered by enzymatic processing of the agent, binding of the intracellular messenger and transfection of the selected plasmid. These agents provide 3D MRI images of gene expression based on intracellular messenger concentration and these are an important physiological supplement to the anatomical role of MRI. This technique can be used for labelling regions that are positive for β-gal expression in the living embryo and for MRI imaging of Ca^{2+} in *Xenopus* cells.

Ching Tung (Massachusetts General Hospital, Boston, MA, USA) presented information regarding amplificable fluorescent probes that can be used for *in vivo* imaging. This method uses an optical imaging approach to visualize activated proteases *in vivo* using quenched near-infrared fluorescent (NIRF) probes that become detectable only after enzymatic activation[10], enabling this method to be used as a gene therapy reporter. Sub-millimetre-sized tumours can be detected, *in vivo*, by lysosomal-sensitive probes with a 12-fold target–background signal ratio. This approach has potential applications for tumour detection, tumour characterization and *in vivo* evaluation of anticancer therapies. Optical *in vivo* imaging can also be used to study brain function following strokes by using an intrinsic contrast to haemoglobin, and for molecular imaging by using an external contrast.

The use of molecular biophotonics for *in vivo* monitoring of gene expression was discussed by Douglas Kawahara (Xenogen Corporation, Alameda, CA, USA). This is a technique based on detection and quantification of light transmitted through living mammalian tissues and production of biochemical light from the luciferase genes that can be cloned and moved from site to site. Light can penetrate up to 10 cm into living mammalian tissue and there-

fore, labelled bioluminescent pathological cells can be tracked in the body[11]. This technology will have a significant impact on monitoring diseases in the patient as well as their response to drugs and other treatments including gene therapy. Furthermore, it improves the predictability of the animal models and can be applied to drug development to reduce the cost and time to product approval.

Kleanthis Xanthopoulos (Aurora Biosciences, San Diego, CA, USA) described real-time monitoring of differential gene expression in live cells using fluorescent probes. The use of miniaturized cell-based screening assays with robotics accelerates the development of novel compounds. Hence, the company's ultra-high-throughput screening system (UHTSS™) enables the screening of at least 10,000 compounds per day.

This was followed by Sonia Connaughton (PE Tropix, Bedford, MA, USA), who described the Xtreme Screen™ system, a chemiluminescence-based high-throughput assay. This assay incorporates 1,2-dioxetane alkaline phosphatase substrates in an immunoassay format for the detection of phosphorylated products of kinases. In protein kinase activity assays, the quantity of light measured is proportional to kinase activity. The use of chemiluminescence technology results in a sensitive, robust and high information-content assay, whereas existing assays can be converted to a miniaturized format without loss of sensitivity or dynamic range.

Concluding remarks

The quality of the presentations at the conference was of the highest calibre, in keeping with the tradition of Cambridge Healthtech conferences. It also provided an important podium for interaction between industry and academia. The dominant theme of this conference was the potential application of novel technologies in molecular diagnostics, and this is discussed in detail in a report on this topic[12]. Many of these technologies also have applications in genomics-based drug discovery[13].

The current trend is the miniaturization of various devices which extends to all their applications. There is also a trend in seeking low-cost *in vivo* diagnostic alternatives to the currently used imaging methods such as MRI. Optical *in vivo* imaging represents one of the biggest growth areas in medical imaging because it is non-invasive and provides real-time, as well as microscopic, imaging. It appears unlikely that the novel *in vivo* sensors will displace MRI, which gives important anatomical details, but instead might become useful adjuncts in gathering physiological information.

The impact of these technologies on medical practice will be significant because of the integration of diagnostics and therapeutics in 21st century medicine. Detection of disease predisposition would be a helpful addition in formulating preventive strategies for major diseases. Meanwhile, *in vitro* molecular diagnostics has already demonstrated its usefulness in the detection and follow-up of various diseases. The ability to monitor these diseases *in vivo* will, therefore, further improve these applications.

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K.K. Jain
Bläsiring 7
CH-4057 Basel, Switzerland
tel/fax: +4161 692 44 61
e-mail: jain@bluewin.ch
Web: http://pharmabiotech.cjb.net

In short...

The **Ludvig Institute for Cancer Research** (LICR; Brazil) and the **State for São Paulo Research Foundation** (FAPESP, Brazil) have announced a US$10 million collaboration with **Amersham Pharmacia Biotech** (Little Chalfont, UK) to speed up global efforts to discover cancer-causing genes in human beings. The project will encompass six new Brazilian sequencing centres, each of which will use the high-throughput sequencing system, MegaBACE 1000, to decode approximately 500,000 sequences of DNA by February 2001. With the use of the new technology, ORSETES (devised at LICR), scientists will now be able to normalise the mRNA population and look at the central coding portions of the genes, where previously they have only been able to sequence the gene extremities. The project's manager, Juçara Parra, said 'This is a really exciting development in the fight against cancer and is especially relevant to Brazil, where currently 13% of all deaths are caused by the disease.'