usefulness of decision trees using holographic fingerprints of chemical structures when applied to primary screening data and convincing examples were provided. This technique also required the generation of rules that can be interrogated further for their relevance, and the preparation of the input data, although details of how this was achieved were not given. This presentation indicated that virtual-screening is becoming a reality.

In summary, the conference provided a good overview of the current state-of-the-art drug discovery. Key messages were that:

- The crucial issues in the business are the attrition rate
- Earlier input of pre-clinical parameters will provide better leads earlier
- The technology to mount significant programmes is now available and 'mature'
- Informatics must play a larger role in exploiting the inputs, and pharmacogenomics (possibly based on SNPs) in optimizing the outputs.

Perhaps these conclusions are already well-known, but these conferences serve to bring all the approaches into perspective, enabling companies to benchmark their activities and perhaps to gain more insight into alternatives. Full conference proceedings can be purchased from IBC (UK).

Ian Shaw
Momentum Healthcare Ltd
Temple Court
Cathedral Road
Cardiff, UK CF1 9HA
tel: +44 2920 786 475
fax: +44 2920 786 466
e-mail: shaw@momentumhealth.co.uk

Enzyme links build better antibiotics

As bacterial resistance becomes more widespread, so the need for structurally and functionally novel antibiotics will increase. Synthetically engineered polyketide synthases (PKSs), which are a group of microbial enzymes that produce drugs such as erythromycin, offer hope for the development of new polyketides to combat bacterial infections. However, one problem is that synthetically engineered PKSs are usually chemically unstable, therefore producing low yields. US researchers have now found a solution to this problem of instability by using specific amino acid-linkers to boost PKS productivity.

Polyketides, from organisms such as actinomycetes, have been isolated and studied for more than a 100 years and have produced some commonly used antibiotics (such as erythromycin, oleandomycin and spiramycin), immunosuppressants (such as rapamycin and FK506), the veterinary antiparasitic, avermectin, and the antifungal drug, candicidin. The discovery of the enzyme systems responsible for their bioconstruction has led to a rejuvenation of the field. Many of the PKSs are multienzyme complexes that can be manipulated to act as 'factories' for the production of new synthetic polyketides.

Genetic manipulation of PKS genes

Novel diverse polyketides can be produced simply by manipulating and rearranging the different enzyme modules within the structure using genetic engineering techniques. Domain inactivation, substitution, or addition of modules by genetic manipulation of the PKS genes, have been used to produce artificial polyketides. Meanwhile, the gene fusion approaches, which have been developed by Peter Leadlay (Cambridge University, UK), Leonard Katz (Abbott Laboratories, Queensborough, UK) and Chaitan Khosla (Departments of Chemical Engineering, Chemistry and Biochemistry, Stanford University, CA, USA, and founding scientist and Chairman of the Scientific Advisory Board of KOSAN Biosciences, Inc., Burlingame, CA, USA) have led to the biosynthesis of diverse synthetic polyketide products. Khosla has highlighted that the manipulations usually result in decreased *in vivo* productivity of the polyketides, the reasons for this being poorly understood. However, suggestions for this reduced productivity include structural instability of the engineered protein, suboptimal chemistry within the altered module, or inefficient processing of the synthetic polyketide intermediates by downstream modules.

Combinatorial biosynthesis of polyketides

Khosla and coworkers, together with David Cane (Brown University, Providence, RI, USA), have spent the past few months trying to overcome this problem. Once these genetically modified PKSs, have been stabilized, they can be used more effectively to produce a diverse range of polyketides, some of which might have useful pharmacological properties, e.g. antibiotic activity.

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01355-0

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.

REFERENCES

1 Rajesh, S. *et al.* (1999) *Science* 284, 482–485

2 McDaniel, R. *et al.* (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 1846–1851

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

  PII: S1359-6446(99)01374-4