Penicillins, such as **4**, modified on Merrifield resin were found to be readily cleaved using aluminium trichloride in nitromethane instead of the anhydrous hydrogen fluoride usually employed. Similarly, penicillins on Wang resin could be cleaved under the same conditions avoiding the use of trifluoroacetic acid. This approach now provides an opportunity for the solid-phase synthesis of libraries of these important antibiotic compounds.

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High-throughput screening

HTS and gene chip technology

New strategies for synthesizing complex arrays of DNA on small glass surfaces to generate what are termed 'gene chips' are revolutionizing the diagnosis of genetic diseases and basic genomic research. The gene chip technology may also have a major impact on drug discovery, making possible rapid monitoring of the effects of a test compound on the expression of a single gene, a family of related genes or even a large chunk of an organism's entire gene pool.

Transcriptional assays are some of the most difficult to convert to high-throughput format. Certainly, the classic techniques for monitoring gene expression – northern and dot blot strategies – are too cumbersome for high-throughput screening applications. Gene reporter approaches, in which the regulatory region of a particular gene is hybridized to a reporter gene, such as luciferase, is one method that has been successfully converted to a high-throughput screening format, but it requires the isolation and engineering of every gene of interest and its reinsertion into a convenient cell line. Such manipulations are time-consuming and not always possible or desirable.

Glass wafers

Gene chips are small glass wafers, usually about 1–2 cm^2 in area, covered with a lawn of precisely defined oligonucleotide probes. They are used in a manner analogous to classic blotting strategies – the probes hybridize to RNA containing a complementary base sequence and can be used to monitor the level of expression of a gene with the complementary sequence. What differentiates the gene chip from classic blotting techniques is that the single small chip can contain thousands to hundreds of thousands of distinct oligonucleotides, each one representing a different gene or small domain of a single gene, and the hybridization can be done quickly and quantitatively [see *Molecular Medicine Today* (1997) 3, 384–389 for a complete description of the gene chip].

Gene chip technology can be used to resequence a known gene quickly and to check for alterations in its nucleotide sequence, to determine the presence or absence of previously identified gene mutations, or, of greatest interest for drug discovery applications, to measure the level of expression of thousands of different genes simultaneously in response to a chemical perturbation of the cell. Using this technology, a high-throughput screening operation might be imagined in which, with a single assay, the effects of a defined chemical or a natural product extract could be determined simultaneously on the expression of thousands of different genes, without ever having to isolate, engineer or reinsert the genes of interest into a host cell.

On the down side, the gene chip assay has not yet been automated for high-throughput screening operations to provide for the testing of thousands of chemical compounds or extracts on a daily or weekly basis. But it is only a matter of time before such systems become available. An automated gene chip assay system would allow thousands of compounds or extracts to be quickly assayed for their effect on thousands of different genes, providing a new plateau for high-throughput screening in which millions of data points could be generated each day. Indeed, it would not be surprising to learn that proprietary systems are already in development, or even in use, by pharmaceutical laboratories that have embraced the gene chip technology for drug discovery.

Some examples

Affymetrix, Inc. (Santa Clara, CA, USA) is the major company developing the gene chip technology; they currently have a product on the market, the GeneChip HIV PRT Assay, which is used to monitor the specific mutant strains of the HIV protease in an infected individual. Based upon the results of the chip-based diagnostic test, a physician can prescribe a cocktail of anti-HIV drugs tailored to the specific strain of HIV that infects a patient. Although most of Affymetrix's emphasis is on using the new technology for diagnostic applications, they are also collaborating with pharmaceutical companies, including Merck & Co., Inc. (Whitehouse Station, NJ, USA), Hoffman-LaRoche (Basel, Switzerland) and Genetics Institute, Inc. (Cambridge, MA, USA), to use the gene chip technology for drug discovery.

Another company exploiting the new technology for drug discovery is Incyte Pharmaceuticals, Inc. (Palo Alto, CA, USA). Incyte has a Life Chip Array that contains probes for more than 15,000 different human genes on a single chip. These genes have been selected from Incyte's LifeSeq GeneAlbum™, which is a library of approximately 100,000 cDNA probes for expressed human genes.

Synteni (Palo Alto, CA, USA) is producing GEM, a chip with an array of up to 10,000 different cDNA elements that can also be used quantitatively to monitor gene expression. Synteni aims to eventually market chips that can be used to monitor the entire human genome.

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Nanogen (San Diego, CA, USA) has taken a different approach to the new technology. It is developing small electronic chips in which electric fields are used to quickly and quantitatively hybridize and dehybridize DNA to individually addressable oligonucleotides. The assays are designed to monitor fewer genes but to do so quickly and repeatedly, just what some drug discovery scientists might need for an automated high-throughput screen. Nanogen is collaborating with Becton Dickinson (Franklin Lakes, NJ, USA) to develop instrumentation for its system. They plan to focus their combined technology on the diagnosis of infectious diseases. However, their electronic chip product and accompanying instrumentation will likely provide powerful new tools that can also be applied to drug discovery.

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Emerging molecular targets

Ceramide-mediated signaling and vasodilatation

The lipid mediator ceramide is a relative newcomer to our knowledge of intracellular signaling events. It is generated by the hydrolysis of sphingomyelin in the cellular membranes by the enzyme sphingomyelinase (SMase) and is believed to act as an intracellular signaling agent. A ceramide-stimulated protein kinase and phosphatase have been identified. In addition, ceramide inhibits the activity of protein kinase C. Activation of the ceramide-stimulated signaling pathway is believed to occur in response to cellular stimulation of cytokines such as TNF-α, IFN-γ and IL-1β.

Gradually, data is accumulating that links the generation of ceramide by SMase to various physiological functions. In one recent report, Drs Douglas G. Johns, Heather Osborn and R. Clinton Webb from the University of Michigan (Ann Arbor, MI, USA) show that increased levels of ceramide may be linked to the relaxation of vascular smooth muscle cells and vasodilatation [*Biochem. Biophys. Res. Commun.* (1997) 237, 95–97]. They found that the level of ceramide in vascular smooth muscle cells rose more than 15-fold above basal levels within 20 min following the addition of 0.1 U/ml of SMase to the cell culture media. Moreover, they found that the increased elevation of ceramide – either by addition of a ceramide analog or the addition of SMase – correlated with a relaxation of endothelium-denuded phenylephrine-contracted rat thoracic aortic rings. Much more remains to be learned about the Smase; however, if divergent tissue-specific forms of the enzyme exist, it may prove to be an interesting therapeutic target.

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