Molecular Technology - A Practical Revolution

A. Introduction

1. Many tremendous and exciting advances are taking place within the biological field, facilitated by numerous biotechnological developments and applications. These have enabled scientists to approach old problems in new and innovative ways as well as allowing them to find unique solutions for various biological, medical, and agricultural needs. The developments have already started to make an impact on society. These technological advances have led to the increased sensitivity and specificity of test assays, the miniaturisation of instrumentation and development of point-of-care devices. This has strengthened and encouraged the development of a strong knowledge base on the ground. An example is '$^{35}S/^{32}P$ phospho-imaging gene sequencing' (a technique that uses isotopes to produce rapid and sensitive images), which is now considered a cost effective and implementable technology in several developing countries. In addition, generic molecular and related isotopic techniques such as southern blots, northern blots, two-dimensional electrophoresis, DNA macro- and micro-arrays and others are equally applicable to the medical and agricultural fields.

B. Isotopic Methods

2. Isotopic methods have played an integral part in the development of molecular biology since its inception many decades ago. Despite the emergence of various alternatives with their associated advantages, both non-stable and stable radioisotopes are still used by dedicated users for established applications and still continue to attract new converts. The high sensitivity and low detection limits unique to isotopic labelling were the main driving forces behind the commercial development of more user-friendly equipment. Radioisotopes represent one of the main detection systems used in molecular biology, hence the emphasis given in this report. Nevertheless, it has to be realized that the current advances in molecular technology span the whole discipline, including the development of numerous alternative approaches.

C. Genomics

3. The term 'genomics' was coined some 20 years ago to define the study of genes and their functions. It followed the development of various technologies and instrumentation required to determine the nucleotide sequences of entire genomes, during the late 70s and early 80s. The DNA sequence is often referred to as the 'blueprint of life' and the impact of its elucidation over the last few decades has been compared to that of the discovery of the periodic table in chemistry. Initially, sequence determination was limited to the simplest organisms such as viruses possessing not more than 16 genes. In 1990, the most ambitious scientific endeavour in the history of life sciences was launched, viz. the sequencing of the full human genome thought to contain about 100 000 genes. This enormous task was completed in 2000 at a cost of $3 billion. The full genome sequences of the

rat, mouse and some pathogenic micro-organisms are now also available and similar studies on a variety of other organisms such as cattle, chickens, sheep, goats and pigs are in progress.

4. Following the successful sequencing of the human genome there has been a flurry of activity, mainly by multinational biotechnology companies, to identify and patent genes with possible applications in human medicine and agriculture. This reflects the growing awareness among scientists and investors of the enormous potential within this field, both for further scientific discoveries as well as future financial profits.

D. Molecular Diagnostics

5. One area that has been escalating exponentially is molecular diagnostics with its associated commercialization. The term molecular diagnostics was coined to distinguish diagnostic techniques that depend on gene sequences from the more conventional *in vitro* tests - a field in which the Agency has been particularly active. In the USA, for example, the demand for molecular diagnostic testing has increased by 600% over the past two years. Molecular detection can contribute significantly to improved disease management and control in various ways. The polymerase chain reaction (PCR) has revolutionized this field and is being increasingly implemented in developing as well as developed countries, with significant assistance by the Agency in many cases.

E. Medical Applications

6. There are numerous conventional laboratory diagnostic assays for human infectious diseases that are often slow, relatively insensitive, non-specific, require invasive clinical procedures and often do not provide quantitative information. In the case of *M. leprae*, *M. tuberculosis* and *Chlamydia*, the infectious agent is either uncultivable or difficult to culture. Molecular detection methods can provide not only definitive identification but also differential diagnosis of the etiologic agent(s), as with respiratory infections due to *Legionella* and *Mycoplasma pneumoniae* where clinical features and serology are uninformative. This information is essential for the prompt institution of appropriate treatment. In the case of *Herpes simplex* and *Chlamydia trachomatis* infections, PCR-based detection is currently being considered as the possible gold standard. In the human health sector, genes or gene groups have already been identified that are involved in genetic diseases such as cystic fibrosis. For thalassemia, out of a spectrum of 200 mutations about 10 are responsible for 90% of the disease in any one country. In Cyprus for example, simplified diagnostic algorithms have been established and national genetic counselling programmes have been established.

F. Livestock Applications

7. In animal health also, the partial sequencing of the genomes of important pathogens (‘gene sequencing’ as opposed to ‘genome sequencing’) is already widely used to study the epidemiology of disease outbreaks, by inter alia determining the origin of infection. This has contributed significantly

to limiting disease spread, as was the case with recent foot and mouth disease outbreaks. In addition, PCR can differentiate between the S19 *Brucella abortus* vaccine strain and wild-type organisms within hours compared to the classical microbiological test, which could take several weeks. Similarly, micro-satellite analyses are used extensively in parentage verification, for the identification of individuals, in the study of population genetics in various animal and plant species and in the conservation of livestock breeds or wildlife species. Genome sequencing of infectious agents has also served as an aid in understanding the causes of disease, where epidemics originate and in improving their control. The detection of drug resistant strains of *Mycobacterium tuberculosis* is being effectively achieved using PCR-dot blot hybridization and ^{32}P radiolabelled probes, or micro-arrays that allow for the batch analysis of samples. In South Africa, 90% of all multi-drug resistant tuberculosis (MDR-TB) cases can be detected in five days by screening for just three codons directly from sputum, as opposed to conventional methods that can take eight weeks.

8. The identification of genes responsible for natural resistance to diseases could lead not only to an improvement in agricultural resource management, but also in the selective breeding and cloning of resistant animals and plants, thereby enhancing or replacing conventional methods of disease control. One of the most important trends with an added market potential is termed molecular breeding, i.e. the application of genomics to the breeding of animals and plants with desired genetic traits. The natural resistance to specific diseases, found in individual animals or local breeds can be used to develop resistant breeds for example. Several genes and traits have already been identified and sequenced and can be used for this purpose. It has been shown, for example, that the incidence of resistance to brucellosis in a cattle herd can be raised within one generation from 18% to almost 53.6% by breeding with parents that tested positive for the ‘brucella resistance gene’. Total resistance may not be achievable, but partial resistance would enhance effective disease management. Another area of potentially important significance is an improvement in the utilization of low-grade forage by ruminants through the transfer of specific genes to ruminal bacteria, which could have important agricultural and economic impacts in certain marginal areas.

G. Biochips

9. In the health sector, the most important application will be the development of diagnostic kits capable of detecting multiple infective agents in a single, highly sensitive, specific and fast assay e.g. biochips. Such tests can also be adapted for field use that would reduce costs considerably, always an important consideration in veterinary diagnostics. The high sensitivity of such assays also means that an infection could be diagnosed in an animal before any symptoms are seen, a great advantage where an epidemic needs to be controlled. In addition, due to its specificity and sensitivity, molecular technology lends itself readily for early warning systems. It is anticipated that a single biochip could be developed for each species, allowing for the detection of all relevant infectious agents. At least one pharmaceutical company is actively pursuing the development of a ‘backpack’ laboratory for field use especially in developing countries. A further refinement combining molecular diagnostics with micro-array nanotechnology, allows for the simultaneous detection of deviations in thousands of genes using a single glass ‘chip’ with immobilized probes and fluorescence-based detection. This technology, however, remains out of the reach of most diagnostic laboratories due to the prohibitive cost of equipment (about $200 000). In such settings, a relatively cost-effective method using nylon macroarray chips and radioisotopic (autoradiographic) detection using phosphor-imaging, is a viable alternative. This is amenable to small-scale automation.

H. Biopharmaceuticals

10. By studying the genetic basis for the individual variation in response to specific drugs, the most suitable therapy for an individual can be selected to avoid unwanted side-effects. Such information can also be used in drug discovery and in developmental strategies such as the design, development and production of new or improved biopharmaceuticals for the treatment of diseases. Despite its tremendous potential, the molecular design, development and production of biopharmaceuticals has not been fully realized as most existing recombinant products are naturally occurring human proteins. Of these, only a growth hormone has found wider application as a stimulant of milk production in dairy cattle. Some progress is being made in achieving acceptance in the community, as seen with the approving of an antisense oligonucleotide drug, fomivirsen, for the treatment of cytomegalovirus retinitis, an opportunistic infection associated with HIV/AIDS.

I. Scintillation Proximity Assay

11. Scintillation proximity assay (SPA) was commercially introduced in 1991 as a rapid and sensitive assay with the capability of high-throughput screening of a wide variety of molecular interactions within a homogeneous system. It utilizes microscopic beads containing a scintillant that can be stimulated to emit light. This stimulation event only occurs when radiolabelled molecules of interest (^{3}H or ^{33}P) are bound to the surface of the bead. SPA is a versatile system and applications include analyses of receptor-ligand binding, enzyme assays, radioimmunoassays, protein-protein and protein-DNA interactions. It is used widely by the drug industry for drug design and discovery. Another important application of SPA is in the detection of enzyme activity. The basic assay involves substrate capture on SPA beads. If an enzyme acts on this substrate, it leads to addition or removal of the radioisotope, which results in a corresponding increase or decrease in signal from the bead. Applications include the screening of drugs that target enzyme function e.g. to screen anti-viral drugs that inhibit viral enzymes.

J. Nuclear Magnetic Resonance

12. Nuclear magnetic resonance (NMR) spectroscopy uses high magnetic fields and radio-frequency pulses to manipulate the spin states of nuclei - including the stable isotopes ^{1}H, ^{13}C and ^{15}N. A NMR spectrum can be obtained of a molecule incorporating such nuclei and can then indicate its structure. The accuracy of NMR spectroscopy for protein-structure analysis is comparable to that of X-ray crystallography. NMR-based screening has become an important tool in the pharmaceutical industry, especially for methods that provide information on the location of small molecular binding sites on the surface of a drug target. In this regard, NMR and X-ray crystallography act synergistically.

K. Vaccines

13. Prevention and control of disease epidemics is a more cost-effective strategy than treatment. In addition, due to developments in the field of molecular epidemiology, improved management of disease is now possible. There is a great need in the market for more user-friendly vaccines that are safer, more heat-stable, confer lifelong protection and are also cost-effective. Improved delivery systems for vaccines as well as more effective adjuvants are also required to achieve this. Much effort is being expended at present on the generation of heat-stable vaccines, for example against Newcastle disease, a devastating disease of chickens especially in developing countries.

14. The identification of specific genes of microbial and viral pathogens that encode for immunogenic protein antigens (proteins) is an important goal for the development of improved vaccines. One approach is to identify the antigens responsible for stimulating the formation of an antibody response after infection, then identifying and isolating the responsible genes and using these to produce recombinant or subunit vaccines. The increasing presence on the market of such vaccines for human use (e.g. the hepatitis B recombinant vaccine) indicates that once current problems are resolved, the genomic approach to vaccine development will dominate the market. Although a recombinant vaccine for rabies has been successfully used in France to eradicate fox rabies, no recombinant vaccine has yet reached the veterinary market in the developing world. A heat-stable chicken-specific poxvirus recombinant vaccine, carrying the gene responsible for conferring protection against Newcastle disease, is presently being investigated for its application in developing countries.

L. Gene Therapy

15. The most futuristic application of biotechnology is probably gene therapy. There are many exciting possibilities, but so far only a few experimental attempts to replace or repair defective human genes have been reported. Further experimentation in animals will probably precede the application in man. It is envisaged that inter-gene and intra-gene manipulations will play the predominant role in this field. The feasibility of gene therapy and its impact will be driven by its uniqueness and its potential for use as in an only option scenario. For most situations, however, the biotechnological revolution will be driven by the need to have better and safer solutions to combating diseases, and for improving the livelihoods of people. It is foreseen that biotechnology will have a major impact on market trends in the future. Analysts believe that the first area to benefit from these developments will be continued diagnostic applications, while market opportunities for therapeutic applications using gene therapy is probably still some way off.