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Leading article

The molecular revolution – coming your way soon

The face of molecular biology is rapidly changing from that of an exclusive scientific club using complex, expensive high technology to a simpler, cheaper discipline which can be routinely employed by the gastroenterologist. Why is this and why now? There are two main reasons. Firstly, molecular biology has begun to deliver its promises of the 80s by identifying genes that are fundamental to human diseases. For example, the genes defective in cystic fibrosis,[1 2] neurofibromatosis,[3] and colorectal cancer[4-8] are known or are beginning to be established. Secondly, alongside these advances a revolution has occurred in the methodology of molecular biology with the development of the polymerase chain reaction (PCR).[9 10]

PCR has been described by Alec Jeffries as 'the molecular biologists' answer to photocopying'. It allows the generation of millions of exact copies of specific pieces of nucleic acid and has changed the practice of molecular biology in the short period since its introduction. Molecular biologists were previously limited by the minute amounts of the gene of interest when compared with the large excess of the background DNA. The choices which faced them were either to clone and replicate the gene of interest in bacterial hosts or to use sensitive radioactive identification methods such as Southern or northern blotting. For many situations these have now been superceded by PCR based techniques which enable new approaches to these problems and produce results in hours or days rather than weeks. Importantly, it is no longer necessary to beg, borrow, or buy a range of probes before starting new work, you can synthesise the primers yourself or order them from within your institution or hospital at no greater price than a monoclonal antibody. Furthermore, as soon as an exciting advance has been reported it is possible to reproduce rapidly the experiments within your own laboratory. The transfer of knowledge no longer takes months or years but days. The cystic fibrosis gene 3 base pair deletion[1 2] is an excellent example, in that within weeks of its description, secondary publications were blossoming within the letter columns of the *Lancet*.[11-13]

How does this technique work? Firstly, the area to be amplified on the gene of interest must be studied to identify two lengths of DNA, usually 20 base pairs long, that surround this area. Complimentary sequences (primers) are then built from the individual deoxynucleotides on a DNA synthesiser. The primers are the key to the reaction as they guarantee its specificity. The DNA is firstly denatured to single stranded DNA by heating to 95°C. The primers bind to their complementary sites when the temperature is reduced to around 55°C, forming two small areas of double stranded DNA. This acts as a template for DNA synthesis when the temperature is raised to 72°C. The DNA polymerase, usually Taq polymerase, then begins to make double stranded DNA starting at each primer site producing two copies of the site of interest. How does PCR generate millions of copies? The simple answer is to repeat the whole reaction 20–40 times obtaining an exponential accumulation of the gene of interest. The reaction product is now so rich in amplified DNA that when run on a simple agarose gel a band of DNA of identical length to the distance between the primers appears, confirming the presence and size of the gene in the original DNA. Thus, someone with cystic fibrosis would have an amplified product three base pairs shorter than a normal person because of the three base pair deletion, and an individual heterozygous for the gene would produce two separate bands.

The sensitivity of the technique is exquisite, with detection limits of a single cell,[14] virus, or bacterium, unlike other molecular biological techniques. Furthermore, the quality of DNA present can be very poor. This means that DNA can be amplified from most clinical specimens, paraffin embedded material,[15 16] and even from paleological material as old as 17 million years.[17]

How will PCR revolutionise gastroenterology? Four areas will initially feel the impact of this technique – microbiology, oncology, genetics, and immunology. These areas will be developed in more detail in the rest of this series, but will be briefly touched upon here.

Microbiology

The diagnosis of viral infection is often by serology and less often by the direct detection of the virus by time consuming or expensive tests. PCR allows the rapid (within five hours) and definitive diagnosis of the presence of a particular virus, and even its typing. The material can be from a variety of sources, and even contaminated with other organisms. Faecal detection of organisms has been made much easier,[18] but more work is required to identify the optimum extraction methods. PCRs to over 20 different viruses have been

This is the first of a series of leading articles on the role of molecular biology in gastroenterology. The editorial committee is most grateful to Dr Philip Quirke, the series editor, and his contributors for their timely contributions to the journal.

developed, many with a direct gastrointestinal interest such as hepatitis A,[19] B,[20] and C;[21] enteroviruses;[22] adenoviruses;[23] human papilloma viruses;[24] measles etc.[25]

It is also possible to diagnose bacterial infections rapidly by the demonstration of bacterial DNA, avoiding culture and biochemical analysis. This technology has the greatest value in detecting the presence of mycobacteria such as *Mycobacterium tuberculosis*,[26] *M paratuberculosis*,[27 28] and *M leprae*,[29] where it is possible to avoid six weeks or more of culture. PCR also allows the direct demonstration of organisms such as *Treponema pallidum*.[30] If the presence of a bacterial toxin is of more interest than the bacteria itself, then toxin carrying strains can be identified by designing primers specific to the toxin gene, as reported for *Clostridium difficile*[31] and *Escherichia coli*.[32] The presence of antibiotic resistant genes could also be excluded, allowing earlier treatment with drugs to which the organism is sensitive. This technique also allows the search for unknown organisms by the use of universal primers to 16S rRNA genes, which amplify any bacteria in the material to be studied.[33] This can then be sequenced and the organism identified. Specific 16S rRNA primers can also be designed as described for organisms such as *Helicobacter pylori*.[34]

Oncology

In oncology the use of PCR is currently limited to the identification of translocations, monoclonality,[35] and of minimal residual disease[36] before clinical relapse in lymphomas. However, should any of the recently described molecular abnormalities of colorectal cancer (Kirsten-ras,[5] the DCC gene,[6] p53 deletion,[4] the APC gene[7 8]) prove to have value in diagnosis or prognosis then these can be rapidly used. The recent description of the APC gene[7 8] will make genetic diagnosis in this condition much easier and will advance our knowledge of the early stage of colorectal neoplasia. PCR can also be used to search for molecular markers of biological aggressiveness in archival tissue, thus removing the period of follow up required for the assessment of their impact on prognosis.

Genetics

The diagnosis of genetic diseases will be simplified by PCR. The parental origin of chromosomes can be traced by PCR detection of inherited polymorphisms such as variable number tandem repeats, microsatellite tandem repeats,[37] and alu[38] or L1H repeat sequences, thus enabling the risk of a genetic disease to be assessed without exact knowledge as to the causative gene. If the gene has been identified, then direct confirmation can be obtained. In the future it may be possible for the gastrointestinal pathologist to go straight from the haematoxylin and eosin slide or paraffin block to an exact molecular diagnosis of a genetic condition without further sampling being needed.

Immunology

In immunology and organ transplantation, the HLA typing of samples will be replaced by an accurate PCR diagnosis of the genetic sequence of the individual[39 40] and much will be learned of the intricacies of HLA disease associations. The level of cytokine expression can also be determined.[41]

Other aspects of the new technology

A range of diagnostic tests is now available, but this is not the limit of the technology. It can provide relatively large amounts of nucleic acid for direct DNA[42] or RNA sequencing, it can create modified nucleic acid sequences[43] which can be inserted into plasmids or transgenic animals to model genetic abnormalities, and can even be used to engineer genetically antibodies for therapy or diagnostic purposes. New genes can be identified using sequences conserved within gene families such as the tyrosine kinases.[44] It may seem unlikely that these molecular techniques will become routine tools of the hospital laboratory but it should be remembered that DNA sequencing has been taken up by many regional DNA laboratories and today's research techniques can rapidly become tomorrow's routine tool, especially if gastroenterologists are aware of what is on offer from the molecular pathologists.

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