REVIEW ARTICLE

The way ahead for medicine with the genomic code

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How do you convince anyone, let alone the staid medical profession, that scientific biological progress is truly astonishing now — unlike anything that has gone before in scale or impact — that the way we practise medicine, our lives, even our history could be changing dramatically?

Within the last few years even seemingly wild predictions of possible biological progress have been reduced to rubble by the unforeseen prodigious advances that have actually occurred. The first gentle waves of these new scientific eruptions are now breaking on the shores of medical practice, but we expect the full force of the '*tsunami*' before the year 2000.

A review of what has been discovered even in the last 6 months would not be a practical proposition, but selected examples will serve this purpose and I will confine comments to the 'genetic' field where most activity is centred at present.

One development that makes the present situation quite different from 5 years ago is the relative ease with which a genomic code that has already been analysed can be re-analysed in the genome of any individual. The readily accessible technique for gene amplification — the polymerase chain reaction (PCR) — is one of the new tools of the molecular geneticist.[1,2] Using heat-stable DNA polymerases and DNA primers that bracket the region of interest (e.g. 50 - 4 000 base pairs of DNA) any known DNA code can be read out of a gene library — usually this takes the form of DNA extracted from leucocytes, tissue, buccal scrapes, chorionic villi or even hair roots. Even a single cell or single spermatozoon has been successfully tested! The technique works because during each cycle (of synthesis, denaturation of DNA and reannealing) the amount of DNA synthesised doubles (Fig. 1). After 20 cycles about a million-fold increase has occurred ($2^{20} \approx 10^6$). A programmable temperature cycler is used to achieve this result (a robust and inexpensive machine has been designed at UCT.)[3] The heat stability of the enzyme avoids the need for more enzyme to be added each time the DNA is heat-denatured at 95°C before the next round of synthesis, and the high temperatures during synthesis also contribute to the superb fidelity of the copying process.

Many applications have arisen from this simple technique and only a few of the medically relevant ones are discussed.

The genetic code is now being dissected at many levels — from mRNA editing and variations in the transcription of genomic DNA,[4] gene organisation, relationships and interactions, the structure of the actual transcription unit for each gene in the nucleus, e.g. in a cell in a particular phase of differentiation, and the factors that turn genes on or off or bear messages hither and thither from the outside.[5]

Applications of genetics

The deeper we seek for highly conserved genomic sequences the deeper our roots appear to be, and similar patterns of organisation are seen in mammals, lower animals, insects,[5] protozoa, fungi and bacteria. Remember too that many growth regulators were first seen as borrowed genes in viruses, hopefully called 'oncogenes' at the time. The way in which many growth factors were discovered therefore gave rise to the confusing category of 'cellular proto-oncogenes'.

Nowadays the relation between viral and cellular genes is recognised and is one of a number of ways in which a growth control gene can be made unresponsive to external signals.

The fact that genomic organisation, in detail, is so similar when higher animals, e.g. mouse and man, are compared, opened up a 'reverse species genetics' approach to understanding human disease. Many diseases of man have animal equivalents — e.g. type I diabetes, hypertension, epilepsy, muscle disease, atherosclerosis — as does the ageing process, but these conditions, as in man, appear to be multifactorial with a number of separate genes involved in different aspects of the disease evolution. The problem can be tackled now

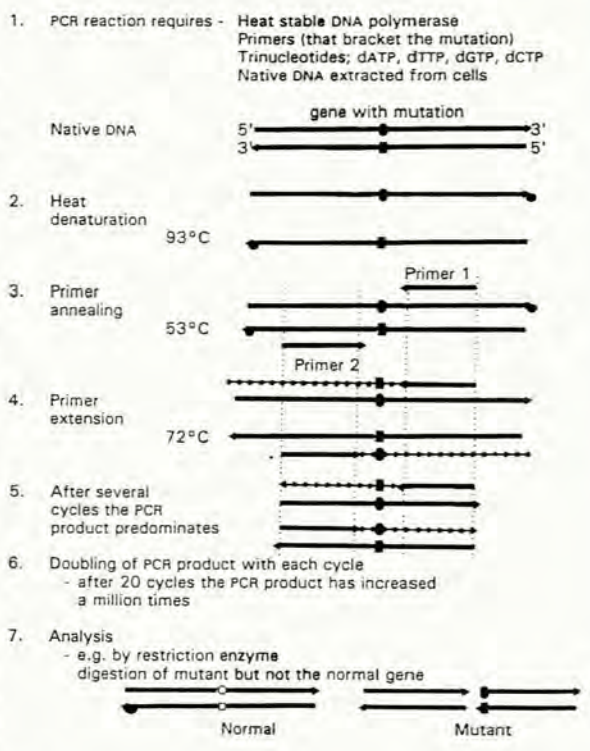

FIG. 1.
Using the PCR to detect a mutation in a gene. Double-stranded DNA is extracted from cells and dissociated by heating into single strands. The specially designed primer sequences complementary to regions above and below the mutated site are added in great excess. When these primers have annealed by complementary base pairing (G ↔ C; A ↔ T) the short region of double-stranded DNA created is used by the DNA polymerase to initiate chain elongation by filling in the complementary bases in the 5' to 3' direction using the trinucleotides as substrate. After a few cycles, a short length of DNA, terminated at each end by the primers, is quantitatively the main PCR product and after 20 cycles the amplification is nearly a million-fold — usually about 30 cycles are used. The temperature cycling is provided by an electronic heating block and the secret of the success of the PCR reaction is the fact that the DNA polymerase enzyme is obtained from organisms capable of withstanding high temperatures.

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by two strategies: (*i*) extremely detailed maps of the mouse genome can be established using a variety of genetically identifiable markers analysed by direct PCR methods; and (*ii*) strains of mice are interbred so that all combinations of the mouse strains, showing the presence or absence of the disease process and its manifestations, are produced. Linkage studies then enable the regions of chromosomes contributing to the disease process to be located and are then redefined, perhaps even at the level of identifying a single candidate 'open reading frame' — in other words the code for a protein. At any point the lessons learnt from mice can be extrapolated to the human family material available. Already at least 3 genes[7] outside the histocompatibility complex, with a role in type I diabetes, have been identified in man by this process. Recently, the gene defect responsible for malignant hyperthermia in pigs was reported[8] and the equivalent defect in humans is to be published soon. The gene involved is for the so-called 'ryanodine receptor', which is the major calcium channel controlling calcium entry into the sarcoplasm of muscle. It will be very good news for anaesthetists if familial malignant hyperthermia can be established genetically.

Products of genes

What can be done when a putative gene product has been identified? The code for the gene can be compared with all known genes through easily accessible data banks. Many proteins have been found to be members of established gene families and to have related functions. However, many relationships will be found that were unsuspected but invite new insights. The cystic fibrosis chloride channel gene,[9] for example, is related to the multi-drug-resistance factor family — food for thought. We can but wonder about our grasp of the subtlety of nature when a neurological disease such as X-linked spinal and bulbar muscle atropy is found to be linked to a mutation in an androgen receptor gene[10] that must therefore have a role in the morphogenesis of the central nervous system. In the last few years literally hundreds of known genes, often previously not amino acid-sequenced, have been completely analysed in the form of reverse-transcribed mRNA, i.e. cDNA or the actual genomic DNA sequence with its flanking controlling regions and introns separating the coding exon sequences.

Organisational patterns are often surprising too! After a long period when the linear bacterial genome was the paradigm, it came as a big surprise to find that human genes were not continuous but broken up into many sections — exons — coding for subcomponents of the protein structure that had to be spliced together to create mRNA for protein translation. The stretches of DNA separating the exons — called introns (intervening sequences) — are poorly conserved with respect to the DNA sequence but highly conserved in terms of position between the exons. We do not really understand why they should exist at all, and perhaps it is the bacteria that are really highly evolved. The antibodies found in SLE were the means whereby the splicing machinery was first discovered, but the relation to the disease process remains enigmatic. Some genes are suspected of having been reimplanted through a mechanism involving reverse transcription of the 'normal cellular' genes of retroviruses. These genes, often non-coding pseudogenes, will resemble mRNA rather than DNA since the exons are already spliced together and not separated by introns as in the original genomic DNA. An mRNA editing function has even been proposed for pseudogenes.

Genetics at the chromosomal level

Chromosome abnormalities have until now been detected by special staining techniques and mainly using metaphase-arrested cultured cells — an insensitive and tedious technique. At least 100 000 base pairs need to be missing before a deletion can be observed in a chromosome under the microscope. Many chromosomal disorders are associated with neurological disease, but this is not surprising in retrospect since at least 60% of all genes are expressed in brain compared with a much smaller range in, for example, liver. Modern techniques such as PCR, using more easily detected probes (also made using PCR technology), enable direct analysis of chromosomal DNA. The fragile X syndrome, a common cause of genetically determined mental deficiency, can now easily be analysed[11] in the symptomless grandfather in whom the gene alteration occurred, followed through his symptomless but transmitting daughter, where the gene is further mutated, and expressed in most males and some females in the third generation as mental deficiency (and usually infertility too). The gene must mutate readily since the disorder is so common — there is probably a connection between this hypermutability (multiple duplication of CpGs) and the fact that the fragile X phenomenon is seen as a fragment of the X chromosome held by a thread of DNA in a fraction of the cells when cultured under conditions of suboptimal nucleotide supply.

Chromosome abnormalities involving translocations can be studied by PCR priming across the breakage region, e.g. in the Philadelphia chromosome, using primers appropriate for two different chromosomes. Chromosome abnormalities could be sought as markers for tumour cell populations in individuals and used to assess tumour burden, e.g. in bone marrow, and response to therapy. We will see a flood of new applications on this plane soon.

New ways to find mutated genes

Genetic lesions occur at more subtle levels, however, ranging from point mutations in inherited metabolic disorders to transposed genes altering gene expression in neoplasms. The present limitations in detecting coding alterations are a consequence of the multitudinous range of mutations that have already been reported even in a single gene, e.g. in lung, liver, colon and bladder cancer in the p53 tumour suppressor gene — a growth regulating factor.[12,13]

Techniques are being developed to look at long stretches of DNA — many thousands of base pairs at a time — to detect point mutations of significance therein, e.g. in factor VIII deficiency, where many mutations occur.[14] If mutated genes are amplified and annealed with a normal gene, mismatches can be detected chemically with specific cleavage reagents. At present this may be academic in the case of a haemophiliac, but probably within a few years exact definition of each mutation will be needed since there is a distinct possibility that mutagenic control of transcription — even mRNA editing — may be an option

Some of these nonspecific techniques for finding mutations involve making large PCR products, cutting them up into pieces of manageable size and looking at the way the single-stranded DNA behaves. Single-base changes often produce different conformations and this results in electrophoretic properties that allow differentiation from normal DNA. The abnormal DNA can then be sequenced readily and the actual mutation or polymorphism defined.

Genes may appear normal, but an abnormal gene product might nevertheless result — this could be due

to deranged control elements, unexpected coding mutations or even mRNA malfunction. In a patient with Duchenne's muscular dystrophy the defective gene product, dystrophin, is produced in muscle and brain and the consequences are expressed in these organs. It is not necessary, however, to use muscle or brain tissue to examine the mRNA (hence the efficacy of transcription from DNA and hRNA conversion to mRNA) since most genes appear to produce a few transcripts in apparently inappropriate tissues[14] and peripheral blood lymphocytes will suffice to demonstrate this 'leaky' transcription. The incredible power of the PCR technique permits this.

At present up to 9 different but common Duchenne's mutations can be sought in a single test tube.[15] The PCR products are tailored for size to be the identifying parameter that is easily analysed.

Population genetics

There are some interesting consequences of our present ability to define the relatedness of individuals more precisely. There have been, for example, embarrassing revelations about sexual mores and some of our idols have failed the acid test — some birds have been regarded as examples of lifelong monogamously paired couples, but on investigation of the progeny a surprising degree of gene 'slippage' has occurred and the contributors have even been identified — as usual unattached males. In some bird harems the doyen is so busy looking after his flock that few of 'his' progeny bear the official genetic stamp.

It is quite possible now to investigate even one's long-departed relatives, e.g. great-grandfather to see if he really was! Only an artefact bearing some of his cells is required — a lock of hair, the stem of a pipe. Lanky US president Abraham Lincoln for example, is being investigated now to see whether he had the gene defect for Marfan's syndrome — a defect in the fibrillin gene — using blood-spattered clothing preserved from his assassination.[16] The implications of a positive finding would be fascinating.

Since genealogies can be authenticated now, maybe the biggest customers for genetic analysis will be probate lawyers and forensic pathologists.[17] There should be no problem sorting out Anastasia at last!

There can be no doubt that the origins, movements and kinships of peoples in the remote past are going to be defined in vastly more detail. History will probably have to be rewritten!

How far back in time are these techniques possible? Human burials' analysed at the Windover swamp site in Miami have established relations between individuals buried 7 500 years ago.[18] But to really impress you, how about fossil magnolia leaves, 18 million years old, analysed for the photosynthetic RUBISCO gene — the commonest protein on earth! The gene compares well with present-day magnolia plants![19]

Genetics, ethics and economics

As is well known, a potent driving force in medical progress — in heart disease, genetic disease and AIDS — is the insurance industry. I venture to predict, despite screams of protest from the 'defenders'of individual liberty, privacy and non-eugenics, that in a few years a catalogue of gene properties — including genes for susceptibility to disease, e.g. HLA types, oncogene status, growth factor status, and actual gene defects, e.g. cystic fibrosis, Tay-Sachs disease, Duchenne's muscular dystrophy, fragile X syndrome, etc. — will be a requirement for determining insurance premium loading and probably a component of normal medical documentation. This is highly likely because PCR techniques become very cheap when performed *en masse* and the technology is ideal for automation. There is however, a lot to learn, about the problems that PCR technology could cause and already intensive efforts to avoid laboratory contamination with PCR products is vital. It is easy to contemplate 100 PCR products tested by allele-specific oligonucleotides even with today's perspectives, and experience has shown repeatedly that our wildest estimates have proved quite timid when looking back only a few years. Could the human genome ever be completely sequenced? 'Never. It would take hundreds of years and millions of dollars!' — however, before the year 2000 all the coding sequences and gene maps will have been done if researchers at the National Institutes of Health have their way — their first dip at brain mRNA has produced many new genes as well as more relatives of known gene families!

Prudent avoidance of genetically determined disease will be a fact of life soon and the way this will affect the culture involved cannot easily be determined. Escaping unpleasant long-term financial problems will be seen by most cultures as a reasonable ploy, but the downside nevertheless is what constitutes 'good or bad' genes and who decides.[20]

The other side of the coin is whether gene therapy, if used to modify genetic disease or to alter normal gene function to produce 'superior' humans, is going to be an acceptable practice. There are many hints already that genes for intelligence exist. There can be no doubt that, notwithstanding attempts at regulation, society will sweep away barriers to obtain any perceived advantage. Merely consider what happened to growth hormone therapy. Creutzfeldt-Jakob prion disease[21] caused the discontinuation of human growth hormone therapy (using human pituitary as source); but recombinant growth hormone has flooded the market as a lactogen for cows and is demonstrably an effective pep-up tonic with minimal side-effects for children with chronic renal failure and for ageing men![22] No doubt once the economics of growth hormone administration in producing giant basketball players are worked out, we will see a sudden taller cohort of players. Maybe adequate regulation will be achieved by moving the goal posts or in this case raising the basketball net so that accuracy of hand and eye is still tested rather than the ability to charge down the court and stuff the ball in the net at eye level!

Gene therapy as an option

Gene therapy may not be as difficult as we currently perceive it to be. There are hints that genes incorporated in viral vectors can transfect cells with the DNA being carried in liposomes — e.g. the bronchial epithelium in the case of α_1 antitrypsin deficiency, and soon perhaps also in cystic fibrosis, where lung disease determines morbidity. As expertise is gained the problem of targeting and expressing the transfecting genes in the appropriate tissue may well be solved. A technique for targeting transfecting DNA to endothelial cells — e.g. in an antecubital vein, where enzymes can be synthesised and released or later ablated if necessary, has already been used. Some lysosomal diseases could be treated in this way. A muscle disease in mice, similar to Duchenne's muscular dystrophy, has been successfully treated by the unlikely process of injecting the transfecting DNA directly into muscle.[23] Transfection of somatic cells with retroviruses bearing the gene for a missing enzyme, e.g. adenosine deaminase in immunodeficiency, seems a realistic proposition.[24]

Gene therapy is of course not only feasible as replacement in cases of genetic deficiency, but could also have a role in targeted anti-sense mRNA production to turn genes off![25] The range of therapeutic options

that this opens up is mind-boggling — animal experimentation is far advanced already.

Diagnostic pathology and the genome

The ability to read the genome is already threatening to have a profound impact on diagnostic pathology.

Where there are distinctive protein products (and therefore mRNAs) or characteristic genomic alterations — deletions, duplications, translocations, maternal or paternal imprinting,[26] predisposing genes,[27] oncogenes, mutated proto-oncogenes, etc. — these can all be assessed in tissue samples. At present the technology for PCR-based detection in the *in situ* hybridisation mode is not worked out, but we can be certain that it soon will be. In the meantime DNA from tissues can be tested, even perhaps with selective micro-sampling. The tissues need not of course be fresh — samples embedded in their wax mausoleums in histopathology departments are also quite suitable. It might well be that a retrospective review of past diagnoses in the light of new information may require the pathology textbooks to be rewritten. Prognosis in neoplasia might not be accurately reflected in morphology or a particular differentiation (protein) product detected by immunohistochemistry microscopically but rather in the variety of cell membrane receptors determining cellular autonomy detected by PCR through their mRNAs. New pathogenic organisms — perhaps HTLV-I in rheumatoid arthritis or retroviruses in Kawasaki disease — may also occur in a range of diseases currently not thought to be related. All we can be sure of is many surprises!

Of course all organisms could be detected by PCR technology — even RNA viruses. The prospect of being able to identify organisms positively and without culture opens up another set of problems and opportunities. The problem of PCR product cross-contamination has reared its head, but solutions are already effective using non-natural, easily destroyed nucleotides in the PCR reaction. The more obvious problems of what constitutes a pathogen and how many organisms constitute a significant infection will have to be solved. Maybe PCR-based technology will solve even these problems by allowing us not only to identify a specific organism but to look for the mRNAs that indicate that the organism is, for example, toxin-producing or in a logarithmic growth phase or sensitive to an antibiotic. The whole ecology of infection and infestation may have to be reformulated. The natural DNA polymorphisms occurring in organisms have also allowed the pinpoint tracking down of the progress of epidemics in communities and aberrations in laboratories.[28]

New fruits of recombinant DNA technology

We are already aware of many products made by recombinant DNA technology — insulin and growth hormone made in bacteria, interferon and erythropoietin made in cultured cells, retroviruses for immunising with a range of bacterial or viral antigens simultaneously, and the numerous proposed techniques for gene transfer by transfection. In this latter technique lie perhaps the only hope for combating AIDS by population immunisation.

Meat from transgenic animals, e.g. pigs, fish, cattle, with improved characteristics such as rapid growth and larger fat-free meat bulk, is already on sale in supermarkets. Another product of more medical interest, however, is the possibility that transgenic animals, e.g. cows, can be created to secrete human proteins — α_1-antitrypsin, anti-haemophilia factor IX, tissue plasminogen activator — in the milk, where they can be harvested and then purified for intravenous administration. An even more intriguing prospect is creation of a milk supply with human lactoferrin as a constituent — this would provide the essential bacteriostatic factor missing from cow's milk infant formulas.[29]

Conclusion

Even with today's perspectives the opportunities for application of our ever-increasing knowledge of the genome seem limitless. The hopeful promises made in the last 20 years are at last being realised in products with a direct impact on patient care.

REFERENCES

1. Eisenstein BI. Current concepts — the polymerase chain reaction: a new method of using molecular genetics for medical diagnosis. *N Engl J Med* 1990; **322:** 178-183.
2. Gibbs RA. DNA amplification by the polymerase chain reaction. *Anal Chem* 1990; **62:** 1202-1214.
3. Purves LR. Robust and inexpensive equipment design for polymerase chain reaction detection of sequence mutations: cystic fibrosis in a mother and 2 children analysed. *S Afr Med J* 1993; **83:** 25-27.
4. Hess JF, Parisi MA, Bennett JL, Clayton DA. Impairment of mitochrondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1991; **351:** 236-239.
5. Hoffman M. New clue found to growth factor action. *Science* 1991; **251:** 1181.
6. Chisaka O, Capecchi MR. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox-1.5. *Nature* 1991; **350:** 473-479.
7. Todd JA, Aitman TJ, Cornall, *et al.* Genetic analysis of autoimmune type 1 diabetes mellitus in mice. *Nature* 1991; **351:** 542-547.
8. Fujii J, Otsu K, Zorzato F, *et al.* Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 1991; **253:** 448-451.
9. Barasch J, Kiss B, Prince A, Saiman L, Gruenert D, Al-Awqati Q. Defective acidificiaton of intracellular organelles in cystic fibrosis. *Nature* 1991; **352:** 70-73.
10. Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991; **352:** 77-79.
11. Yu S, Pritchard M, Kremer E, *et al.* Fragile X genotype characterized by an unstable region of DNA. *Science* 1991; **252:** 1179-1181.
12. Levine AJ, Momand JM, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991; **351:** 453-456.
13. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350:** 429-431.
14. Naylor JA, Green PM, Montandon AJ, Rizza CR, Giannelli F. Detection of three novel mutations in two haemophilia A patients by rapid screening of whole essential region of factor VIII gene. *Lancet* 1991; **337:** 635-639.
15. Chamberlain J, Gibbs RA, Ranier JE, Caskey CT. Multiplex PCR for the diagnosis of Duchennes muscular dystrophy. In: Innis MA, Gelfand DH, Slinsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications.* Emeryville, Calif.: Academic Press, 1990: 272-281.
16. McKusick VA. The defect in Marfan syndrome. *Nature* 1991; **352:** 279-281.
17. Hagelberg E, Gray IC, Jeffreys AJ. Identification of the skeletal remains of a murder victim by DNA analysis. *Nature* 1991; **352:** 427-429.
18. Lawlor DA, Dickel CD, Hauswirth WW, Parham P. Ancient HLA genes from 7 500 year old archaeological remains *Nature* 1991; **349:** 785-788.
19. Cherfas J. Ancient DNA: still busy after death. *Science* 1991; **253:** 1354-1355.
20. Aldhous P. Who needs a genome ethics treaty? *Nature* 1991; **351:** 507.
21. Weissmann A. A unified theory of prion progogation. *Nature* 1991; **352:** 679-683.
22. Rudman D, Feller AG, Nagraj HS, *et al.* Effects of human growth hormone in men over 60 years old. *N Engl J Med* 1990; **323:** 1-5.
23. Acsadi G, Dickson G, Love DR, *et al.* Human dystrophin expression in mdx mice after intramuscular injection of DNA constructs. *Nature* 1991; **352:** 815-818.
24. Ferrari G, Rossini S, Giavazzi R, *et al.* An *in vivo* model of somatic cell gene therapy for human severe combined immunodeficiency. *Science* 1991; **251** 1363-1366.
25. Moffat AS. Making sense of antisense. *Science* 1991; **253:** 510-511.
26. Little M, Van Heyningen V, Hastie N. Dads, disomy and disease. *Nature* 1991; **351:** 609-610.
27. Bandara LR, Adamczewski JP, Hunt T, La Thangue NB. Cyclin A and the retinoblastoma gene product complex with a common transcription factor. *Nature* 1991; **352:** 249-251.
28. Chermann J-C, Waters D, Hall L, *et al.* Sequence analysis of original HIV-1. *Nature* 1991; **349:** 745-746.
29. Gershon D. Will milk shake up the industry. *Nature* 1991; **353:** 7.