Australian Orthoptic Journal 1990; 26: 45-47

AN UPDATE IN GENETICS FOR THE ORTHOPTIST A BRIEF REVIEW OF GENE MAPPING

L ADAMS, DOBA, DipAppSci(Cumb)
Retinal Dystrophy Service, Prince of Wales Hospital

Abstract

Genetics in the field of medical research is an area which is rapidly developing. The process of mapping the human genome via the method of reverse genetics is resulting in the chromosomal location and identification of genes responsible for various inherited disorders. The specific location of genes on specific chromosomes will allow for prenatal diagnosis, presymptomatic diagnosis, carrier detection, confirmation of individuals affected and, later on, possible treatment. In some disorders this has already been the case.

This paper gives a brief overview of one of the techniques used in gene mapping known as Recombinant DNA Technology in relation to inherited eye disease.

Key words: *Recombinant DNA technology, molecular genetics, reverse genetics, chromosomal locations, inherited eye diseases.*

INTRODUCTION

Hereditary conditions until recently have been diagnosed largely on a descriptive basis. This included the pattern of inheritance (family trees), clinical evidence and the natural history of the disorder. It is now, however, possible to describe and diagnose these conditions on a molecular (genetic) basis via reverse genetics and chromosomal locations.

In 1979 the editor of the American Journal of Human Genetics, David Coming, described a new approach to gene mapping and referred to it as the "new genetics". There are today many methods of gene mapping, of which recombinant DNA technology is only one.

To date there has been in the vicinity of 1,200 autosomal genes localised, and 150 X-linked genes mapped.[1] These advances are now playing major roles in the areas of prenatal diagnosis, presymptomatic diagnosis, carrier detection, confirmation of an affected individual and treatment.[2]

Medicine is becoming more and more involved in genetics with these new procedures. The advances made in the treatment of various diseases and the new technology available has meant that many conditions have "cures", but it is the genetic conditions that are still a mystery in many areas of medicine. It has been estimated by Burn[1] that when single gene disorders, chromosomal disorders and structural malformations are grouped together, that they will account for half of all miscarriages, a quarter of perinatal deaths and three-quarters of severe handicaps. As well, one in eighty adults will be affected by a late onset genetic disorder, ranging from Alzheimer's and Huntington's Chorea to diabetes and heart disease. The area of ophthalmology and inherited eye conditions is no different, with genetic disorders ranging from

Address for correspondence: L Adams, School of Orthoptics, Cumberland College of Health Sciences, East Street, Lidcombe, NSW 2141

retinoblastoma to colour blindness, aniridia and retinal dystrophies. Thus it is the responsibility of all clinicians (including orthoptists) to be aware of the new technology in genetics so that it can be used to the best advantage and care of our patients, especially when it comes to counselling and the services available to them.

REVIEW OF THE CELL AND ITS GENETIC MAKE-UP

In order to understand the process of gene mapping and recombinant DNA technology, it is necessary to review the components of the cell and its genetic make-up.

The nucleus of the cell contains lengths of deoxyribonucleic acid (DNA). These are known as chromosomes, which are grouped into 23 pairs, 22 pairs of autosomes and one pair of sex chromosomes. The DNA consists of a phosphate and sugar band with nucleic bases, bonded loosely together in pairs. There are four bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T). These bases are always paired: A = T and G = C. A specified sequence of these bases will lead to the production of a protein.

It is on the chromosome that the genetic information is stored. A gene therefore is a length of DNA containing the information for a specific protein. These specified sequences are not continuous and are known as exons. The intervening sequence which breaks the code between genes and the proteins they produce are known as introns.

The chromosomes, being loosely bonded, separate easily and become single stranded in the process of cell division, providing templates for two new molecules. The DNA after separating is complemented by a matching single strand of ribonucleic acid (RNA), only the base Uracil (U) is used instead of Thymine (T).

These 23 pairs of chromosomes, consisting roughly of six million base pairs, with more than 50,000 genes, constitute the Human Genome. There is a huge task involved in mapping it and it will take many years before it is completely mapped.

GENE MAPPING — RECOMBINANT DNA TECHNOLOGY

Before discussing the procedure involved in locating the chromosome and therefore an abnormal gene, further definitions of new terminology are required.

1. Restriction Enzymes: These are enzymes called restriction endonucleases. When a specific enzyme is applied to DNA it will read and cut the DNA at a specific base sequence, thus producing fragments of DNA of different lengths. The recognition sites vary in length from 4-8 base pairs.
2. Restriction Fragment Length Polymorphisms (RFLPs): These are normal variations in the base sequences of DNA They are inherited and have no apparent clinical effect.[2] These polymorphisms can be detected by subjecting DNA to a restriction endonuclease which will produce a specific length of DNA This is known as a Restriction Fragment Length Polymorphism (RFLP).[3]
3. Gene Probes: A gene probe is an identical copy of a sequence of a single strand of DNA which has been cloned. The copy can be made from messenger RNA by the enzyme reverse transcriptase or directly from DNA The result is a complementary strand of DNA (cDNA). The cDNA also has a radio-active base known as p^{32} included in its phosphate-sugar band. This addition of p^{32} allows the probe to be located by autoradiography and therefore aids in the detection of abnormal genes.

PROCEDURE INVOLVED IN GENE MAPPING

DNA is obtained from any available tissue. The most common and easily accessible form is a blood sample of at least 5 mls. This is sufficient to analyse any normal or mutant gene for which the probe is available. The white blood cells are then extracted, which contain a high proportion of DNA material.

A restriction enzyme of choice, depending on the chromosome and region under analysis, is then added, generating fragments of DNA of specific lengths. These fragments are then

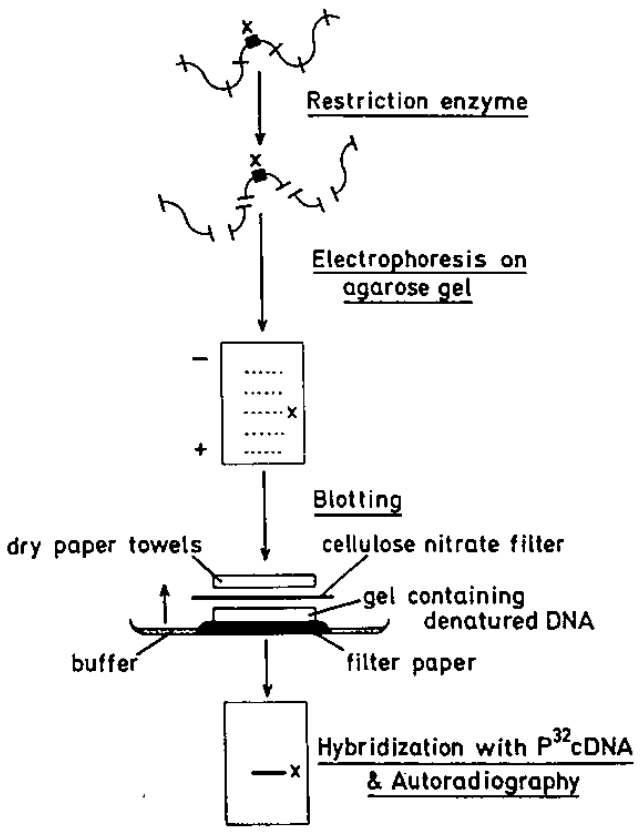

Figure 1: Showing the technique of Recombinant DNA Technology (from Emery AEH. Elements of medical genetics, 6th ed. Edinburgh, Churchill Livingstone, 1983).

subjected to a process known as electrophoresis in agarose gel. This separates the fragments of DNA, which migrate distances according to their molecular weight. Smaller fragments migrate faster than larger ones. The DNA then undergoes a process of denaturisation by an alkali which separates the double strand of DNA into a single strand. This makes it capable of bonding to a probe (cDNA). The single strands are then transferred to a cellulose nitrate filter by a process known as "Southern Blotting".

Next, is the addition of a radioactive probe to the filter. When the cDNA finds its complementary sequence on the filter it 'bonds' and a double strand of DNA is formed. In order to detect an RFLP, a probe within the restriction site of the polymorphic region must be used. The position of the probe can be localised and visualised by auto-radiography and any abnormalities noted.

The radioactive bands highlight the variations and appear at different places as blots of DNA from different individuals. A single blot will indicate that the person is homozygous and a double blot heterozygous.[4] In some cases the band may be missing indicating an abnormality (Figure 1).

SUMMARY

This in a 'nutshell' is a brief description of gene mapping via recombinant DNA technology which is used to localise genes responsible for inherited conditions.

It is hoped that this brief article has helped bridge the gap a little in regard to this rapidly developing area of medicine and genetics.

ACKNOWLEDGEMENTS

I wish to thank Dr F B Halliday and Mrs C Toohey, without whose help and support this paper would not have been possible.

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