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appendix 1

Context and process

Section contents

2.3 Genetic modification technology and its use in New Zealand

Introduction

Fundamental to genetic modification is deoxyribonucleic acid (DNA). DNA provides the information by which cells know what to do, what to be, and how.

DNA is found in cells, specifically in the nucleus, where it is found in giant complexes called chromosomes. DNA is a long strand of joined molecules called nucleotide bases, of which there are four: adenine, cytosine, thymine and guanine. Two strands of nucleotide bases are usually bound together (adenine bonding with thymine, cytosine with guanine) to form the famous double helix.

The sequence of bases is important because it provides the code for proteins (including enzymes) or, in some instances, ribonucleic acid (RNA). (RNA is a single strand of the nucleotide bases found in DNA, except that uracil substitutes for thymine. RNA differs from DNA in that the sugar ribose, and not deoxyribose, forms part of its structure.) To produce a protein, the part of the DNA which codes for it, the gene, is used as a template to make a strand of RNA. The RNA moves out of the nucleus into the cytoplasm of the cell where it is used to bring protein 'building blocks' (molecules called amino acids) together in the correct order, according to the code, to construct the protein. Therefore it can be said that genes are expressed as proteins.

Genetic modification is the manipulation of the DNA of an organism by adding or subtracting bases in the DNA sequence, or by adding or subtracting entire genes to or from the sequence. A variety of procedures allow this kind of manipulation. This section provides a basic introduction to some of these procedures and their applications in New Zealand. The use of genetic modification and its technologies in New Zealand is, however, widespread and diverse, and the examples below are by no means exhaustive.

Many of these examples are drawn from information provided to the Commission by Interested Persons in their submissions and witness briefs. These are referenced by the 'IP' number assigned to each of the submitters. (Full details of the

Interested Persons nomenclature is available in Table 1, Appendix 2.) A name follows the number when the information is drawn from a witness brief. The Interested Persons referenced in this section are listed by IP number in the "References and further information". All the submissions and witness briefs are publicly available on the Commission website (http://www.gmcommission.govt.nz) until 30 June 2002.

A glossary of technical terms forms part of the "Glossaries" section later in this volume.

Basic techniques of genetic modification technology

Genetic modification technology employs several major techniques, which can be used alone or in combination:

- DNA fragmentation
- recombination
- transformation
- selection
- cloning
- DNA libraries
- identification
- sequencing
- restriction mapping
- insertional mutagenesis
- DNA injection
- knockout technology and homologous recombination.

Much of the following description of the techniques is based on that provided by Strickberger (1985). Other descriptions are drawn from NHGRI Glossary of Genetic Terms and Interested Persons ([IP59] Morris, [IP13] Woodfield). Descriptions of knockout technology and homologous recombination are derived from websites listed at the end of this section (see "References and additional information").

DNA fragmentation

Fragmentation of DNA allows technologists to work with the genes of an organism more easily than if the genes were in their natural state. Usually, only a

few genes or sequences of a genome are of interest or able to be dealt with. It is therefore necessary to fragment DNA that contains these genes. DNA fragments are created either by exposing the genome to enzymes (often restriction enzymes) that cut the DNA at known base sequences, or by various mechanical shearing techniques. The resultant fragments will be of different lengths and sequences, and may or may not contain the gene(s) of interest. Further manipulation is required to isolate the target sequences.

Recombination

The process of recombination involves bringing DNA from different sources together. Usually the sources of the DNA are the fragments, some of which may contain the genes of interest, and vector DNA, which allows the transportation of the gene of interest into a host. This is usually done for one of two reasons:

- replication of the recombinant DNA to provide many copies of the gene, or
- translation of the gene into its product (ie, a protein).

This process is often done using plasmids. (A plasmid is a circular DNA molecule, not associated with a chromosome, which can replicate autonomously.) Plasmids are particularly common in bacteria and are often associated with antibiotic resistance.

To create recombinant DNA, a specific plasmid is selected and copies of it are cut at a specific site, frequently also with a restriction enzyme. This produces an opening in the plasmid ring. The open plasmids are mixed with the DNA fragments created earlier, and enzymes that allow DNA to rejoin. This technique produces plasmids that may have a foreign fragment of DNA inserted into them. (There will also be plasmids that have no inserted DNA.) Of the plasmids that contain one of the foreign DNA fragments, only some will contain the DNA of interest.

There are numerous variations on this method of forming recombinant DNA, using a variety of enzymes. The basics of the process are similar.

Transformation

The recombinant plasmids need to be inserted into a cell for the genes they contain to be expressed. A common way of transforming cells with (recombinant) plasmid DNA is by using chemicals to make the host cell walls (plant, animal or bacterial cells) permeable to the plasmid, allowing the plasmids to enter.

Bacteriophages (viruses that are parasitic in bacteria; also known as phages) may also be used as vectors ('carriers'). Foreign genetic material can be incorporated

into their DNA so that they may transfer this genetic material to a bacterial cell. Using phages, rather than plasmids, allows longer DNA fragments to be manipulated. Once inserted into the phage, these foreign DNA fragments remain more stable than they do in plasmids. Bacteriophages also enter cells more easily than plasmids.

Selection

No matter which of these transformation techniques is used, the frequency of transformation is low. Transformation results in a mixture of cells, some of which will have been transformed with the DNA of interest, some with other DNA fragments and some will not have been transformed at all. Therefore it is necessary to be able to distinguish between these types. This is usually done by ensuring that the plasmid-transformed host cells express a particular, known phenotype (or observable characteristic), and that those that contain the correct DNA fragment express a second known phenotype, which can then be tested for. These phenotypes are commonly, but not exclusively, antibiotic resistance. The genes for these phenotypes are thus 'markers'.

Cloning

Once a host cell, say bacterial, has been identified as containing the gene of interest, it can be grown to produce many bacterial cells. All the bacteria grown will have the same recombinant plasmid; they are called clones. By cloning the bacteria, enough of the gene fragment can be produced so that the gene of interest may be isolated and identified.

Vectors, especially plasmids, can be created so that the foreign DNA can be transcribed and translated (ie, turned into the protein(s) for which it codes) inside the host cell. Cloning enables a large amount of the gene product to be created. This is used when the gene(s) of interest produce a useful compound.

A different cloning technique can be used to produce many copies of a piece of DNA. If a particular, known DNA sequence is desired, the RNA copy of the sequence (mRNA) can be isolated from the cell, and exposed to an enzyme called "reverse transcriptase" which produces DNA copies of the mRNA.

DNA libraries

Gene libraries are used to provide a source of clones of a particular whole genome. To this end an entire genome is fragmented, the phage vectors prepared and recombined with the DNA fragments. An appropriate bacterial strain (often *Escherichia coli*) is infected with the phages so that a stock of cells carrying the entire assemblage of foreign DNA fragments is maintained.

In this way the genome is cloned into hundreds of thousands of phage particles. The result provides a library of random DNA fragments from which particular clones can be selected and identified.

Identification

There are various, related techniques for identifying clones that contain DNA of interest. Radioactively or fluorescently labelled probes (single strands of DNA whose sequence is known) are allowed to mix with a variety of clones whose DNA has been denatured (changed to a single-stranded state). Those with a base sequence complementary to the probe will bind with it and can be recognised by their radioactivity or fluorescent nature. These can then be further cloned, until sufficient amounts of DNA have been produced for further study.

Sequencing

Sequencing is the process by which the order of the nucleotide bases on a strand of DNA can be determined. To do this, copies of the unknown sequence (taken from clones) are radioactively labelled at one end. They can then be broken down using four chemical treatments which remove one or two specified bases. This results in fragments of different sizes containing known numbers of bases, starting with one which was originally labelled. Using logic, the sequence of the bases can be determined.

Since these techniques were first developed, more specific, non-radioactive labelling procedures have been discovered. It is now possible to label or "stain" DNA fragments with any of a number of fluorescent dyes, and to visualise the chromosomal location of one or several probes simultaneously through a fluorescence microscope. Sequencing genes has thus become an automated process.

Sequencing a gene is an initial step in determining the function of genes. It is also important in the location and identification of "markers". Markers are sequences of bases or genes that occur in an identifiable location on a chromosome and enable the location of other genes that may not yet be identified.

Restriction mapping

Restriction enzymes are used to cut DNA at specific (known) sequences. This allows the DNA to be partitioned into segments that can be individually identified by their molecular weight and put into sequential order. Then, only partial digestion is allowed so that larger fragments are obtained. These are digested separately to see which segments they contain. By using overlapping information

the entire DNA molecule can be ordered onto a "restriction map". The map shows the order of the genes rather than the sequence of bases.

Insertional mutagenesis (tagging)

Insertional mutagenesis or tagging is a technique often employed to determine the function of genes in plants. Naturally occurring transposons (mobile genetic elements that can shift from one location in the genome to another) are introduced into a target plant and mobilised in the progeny of the plants. Where the transposable element inserts into a gene, the inactivation of that gene often occurs, resulting in an altered phenotype. Thus "molecular tagging" can occur. The transposon tagging can then be used in identifying and cloning a gene of interest.

DNA injection

A small volume of DNA is injected into a single-cell zygote to produce animals with additional foreign DNA. This approach has been widely used to express a gene of interest in high quantity ("over-express" a gene). It results in random or semi-random integration of the gene, usually into a single genomic site.

Knockout technology and homologous recombination

Gene knockout is the inactivation of a gene in a living organism: it is fundamental for the investigation of gene function, and uses homologous recombination techniques. Homologous recombination (or gene targeting) results in the normal gene being removed from the chromosome and replaced by the inserted one, at the same site on the chromosome. In older techniques, insertion occurred randomly in the genome.

A gene with the desired mutation is made and a selectable marker gene is attached to it. On each end of this construct, base sequences, identical to those on either side of the gene in the organism, are attached. This is then put into embryonic stem cells which have been removed from the host organism and which are grown on, artificially. In some of these cells, normal processes involving enzymes will cut both the host DNA and the inserted DNA at points in the identical base sequences and a swap between these sections will occur. This is homologous recombination and, where it has occurred, cells will express the phenotype of the marker and can be selected.

To create a knockout organism, these cells are injected into embryos which are implanted into female organisms to be born normally. Each offspring will comprise cells that are genetically altered and normal cells. Those that produce

reproductive cells with the altered gene are cross-bred to produce offspring that only have the defective gene, ie, they are knockout organisms.

How long have genetic modification techniques been used in New Zealand?

The first genetically modified organism was produced in a laboratory about 28 years ago [IP19], and genetic modification techniques have been commonplace for at least 20 years [IP77a]. In line with this figure, Environmental Resource Management Authority [IP76] reports that genetic manipulation began in New Zealand 20 years ago, and this technology has become increasingly widespread since that time.

For example AgResearch [IP13] reports using transformed *E. coli* for experimentation for the past 15 years. Institute of Molecular BioSciences (Massey University) [IP15] has also run genetic modification technology workshops for 15 years. Some genetically modified pharmaceuticals have been available in New Zealand for this length of time.

As this technology has become more commonplace worldwide and new technologies have become available, more groups in New Zealand have adopted these techniques in their programmes. Some genetic disease tests that utilise genetic modification technology have been available in New Zealand for the past six years [IP91], while Malaghan Institute of Medical Research [IP10] has been using genetically modified mice for the past five years. Landcare Research [IP12] has used genetic modification technology for the past five years, while Wrightson [IP3] reports that it has been using genetic modification techniques for only the past two years. Imported foods and feeds, which may contain genetic modification products, have been available in New Zealand over the past two or three years [IP56].

Use of genetic modification in New Zealand

The use of genetic modification techniques and products in New Zealand is widespread. Genetic modification technologies are employed by Crown Research Institutes, private companies, universities and medical institutions:

- to identify genes and their functions
- to investigate pest and disease resistance in animals and plants
- to investigate livestock fertility

- to understand, diagnose and treat human disease
- to investigate control of environmental problems
- for educational purposes.

There have been field trials of genetically modified plants and animals, and genetically modified laboratory animals (particularly mice) are often employed for research. The fundamental importance of microorganisms to this type of research means that many transformed microorganisms have been created and/or used in this country. Genetically modified products available in New Zealand mainly involve human medicines. Some imported foodstuffs may also contain genetically modified products.

Animals

Microorganisms

Microorganisms are fundamental to genetic modification technology, as vectors and for cloning DNA. Some are involved in food preparation processes, others in applications in animal, plant or human health. They will be dealt with more specifically in applications described below.

Native animal species

Scientists at such places as Landcare Research and Massey University are involved in using genetic modification technology to identify genetic variation in endangered and other native animal species. This type of research involves modifying *E. coli* strains with DNA from the species of interest to determine characteristics of the genetic sequences. In this way new species of native fish, kiwi, tuatara, skink, squid, peripatus and beetle have been identified ([IP19] Wallis). The mitochondrial genome of the kakapo has been nearly completely sequenced ([IP92] Penny) and the genetic variation of black and bush robins, sex identification of native birds, investigation of genetic diversity in tuatara, investigations into dispersal and mating systems of possums, and the production of markers for use in conservation of a wide variety of native flora has been accomplished ([IP15] Sarre).

Insects

Genetic modification of bacteria from the gut of wasps is being investigated as a means of biocontrol of wasps [IP12]. The intention is to insert a toxin gene(s) into wasp-associated bacteria such that they kill the wasps in their nests. The research has not reached the stage of producing transgenic organisms, but the isolation of possible target bacteria from wasps has been achieved ([IP13] Goldson).

Worms

Transgenic worms have been created by the addition of other worm genes, and fluorescent protein genes to research the development of possum-specific parasites [IP13].

Possums

Landcare Research [IP12] is collecting DNA from possums to identify genetic markers, and to generate enough DNA, by cloning, for DNA sequencing. Researchers are working on finding new ways to control these pests. One strategy is to control the fertility of possums. To achieve this, possum egg coat and sperm proteins have been cloned, sequenced and used to immunise female possums. This involves the creation of genetically modified bacteria and plants that express the possum proteins. Mapping of a recombinant possum protein is under way.

Stoats

There has been work into stoat biocontrol in New Zealand [IP12]. Part of this work has involved using DNA profiling to estimate stoat abundance. Massey University is investigating a vector, in the form of a gut bacterium, for the biocontrol of stoats.

Cattle

Basic work is being carried out to map and sequence the bovine genome. New Zealand Dairy Board [IP67] is hoping that this will lead to improved traditional breeding techniques, by allowing useful genetic characteristics to be identified in individuals, which can then be bred.

More specific research is also being carried out to create transgenic cattle. Two of the genetic modifications are the insertion of additional copies of two cattle milk casein genes and the disruption of an existing cattle milk protein gene. These modifications aim to alter the amount of particular proteins that already exist in milk of the cattle.

The third genetic modification involves the insertion of a synthetic copy of the human myelin basic protein gene into the cattle genome. Cattle carrying this gene secrete the protein into their milk, from which it may be purified and ultimately tested for its efficacy in the treatment of multiple sclerosis ([IP13], [IP34]).

Sheep

AgResearch [IP13] is involved with identifying naturally occurring genetic mutation in New Zealand sheep breeds and developing novel biological products (hormones, vaccines and diagnostics).

Lincoln University ([IP8] Palmer) has projects to characterise variants of a gene involved with increased lamb growth. This gene was fragmented, cloned in a

plasmid vector and used to transform *E. coli*, so that the nucleotide sequences of the different variants could be determined. This allows sheep with favourable variants of the gene to be selected for further breeding using more traditional methods.

A flock of transgenic milking sheep has been established in New Zealand. These sheep produce milk which contains human Alpha 1 Antitrypsin (hAAT), a protein that is used to combat cystic fibrosis ([IP25] Wakelin).

Myostatin, a protein that is believed to restrict muscle growth and development, is the subject of planned research in New Zealand. AgResearch [IP13] and others have determined that the myostatin gene in double-muscled Belgian Blue cattle is defective, resulting in the expression of an inactive form of the protein. In the future, development of a transgenic sheep with an inactive myostatin gene will enable research into sheep with enhanced muscle growth and development. The first stage of the work will involve introducing a synthetic antibiotic resistance gene into the myostatin locus in ovine somatic cells. The antibiotic resistance gene will be used for selecting cells that have undergone recombination. In subsequent stages, a myostatin knockout sheep will be generated by nuclear transfer and cloning technology. These procedures are now well established at AgResearch. Crossbreeding will then be carried out to generate myostatin knockout sheep ([IP13] L'Huillier).

Transgenic sheep modified with a mouse promoter gene so that they overproduce a growth factor hormone believed to increase wool production have also been investigated at Lincoln University. A DNA construct containing the growth factor gene and the promoter gene was injected into sheep embryos, which were transferred to ewes for gestation. A male offspring expressing the transgene was mated to normal ewes, producing many transgenic offspring. These were then investigated for increased wool production over several seasons ([IP8] Palmer).

There have been various genetic modification studies involved with the health of sheep flocks. For example, Lincoln University has studied an enzyme that causes a predisposition in sheep to develop cataracts ([IP8] Bickerstaffe). A modified bacterium, *Bacillus thuringiensis* (Bt), that is active against maggots in sheep flystrike has been produced ([IP13] McNatty).

In other genetic modification experimentation, Lincoln University is studying an enzyme system involved in meat tenderisation. The central portion of the genes for the enzyme system were cloned into a plasmid vector in an *E.coli* host and the sequences of the cloned fragments determined. This information was used to predict the amino acid sequence of the protein involved in the tenderisation

process. This research has indicated that tough meat is the result of low expression of the gene in muscle ([IP8] Bickerstaffe).

Sheep genotypes are also being investigated in relation to developing cures for infertility in humans ([IP13] McNatty; [IP47]).

Mice

In many areas of research, especially medical research, transgenic mice play a key role. Many of these mice are imported. AgResearch at Ruakura is the only centre in New Zealand that currently produces transgenic mice in New Zealand. These mice are either knockout mutants (ie, they have a deletion, or partial deletion, of a gene) or transgenics, which have additional gene(s) inserted into their DNA. Mice are usually modified in these ways to imitate human disease conditions, which can then be studied ([IP45] McLennan).

Animal vaccines

There are various aspects of animal disease treatment where genetic modification technology has been important. For example, a vaccine against feline leukaemia virus (FeLV) that contains a recombinant DNA-derived glycoprotein is commercially available in New Zealand ([IP28] Squires).

As part of the successful eradication of Aujeszkys Disease from New Zealand porcine livestock, the New Zealand pork industry utilised a gene deleted vaccine [IP28]. New Zealand Association of Scientists reports that there has been a hydatid vaccine developed from genetically modified *E.coli* ([IP92] Heath). 'MeganVac' is a vaccine available in New Zealand to prevent *Salmonella* outbreaks in poultry. It is produced using a gene deletion procedure to weaken the pathogen ([IP35] Diprose).

As well as simply using imported vaccines, there are underway in this country various investigations into producing novel vaccines for a range of pathogens. For example, a study into a vaccine against Johne's disease is ongoing, as is an effort to produce a vaccine against *Salmonella* Brandenburg which affects sheep in the South Island ([IP28] Squires).

More research-oriented projects involve ongoing work that will determine the ability of an organism to recognise and produce antibodies to pathogens. New Zealand whales, dolphins, seals, sea lions, black robins and leiopelmatid frogs are being investigated in this way ([IP19] Wallis).

These are only some examples of the types of work using genetic modification technology with animals in this country. Other animals, eg salmon, fireflies and blowflies, are also the subject of genetic modification investigations in New Zealand [IP85].

Plants

Plants are readily manipulated by genetic modification technologies. They can more easily be grown from a single cell than animals. Many plants of economic interest have been investigated and modified, for example forage crops, horticultural crops and forestry plants, and there is also interest in native plant species. Plants may be genetically modified to become pest-, disease- or herbicide-resistant, tolerant of a wide range of environmental conditions, or suitable for use in bioremediation or pharmaceutical production. They may also provide material for research into genetic modification techniques, plant development or gene function.

Genomics and bioinformatics in plant studies

Foundation for Research, Science and Technology (FRST) contributes funds to genomics programmes in organisations such as HortResearch, which has set up genomics projects to identify: genes and proteins involved in plant responses to pests and diseases; genes that control plant development, architecture, flowering and fruit quality characters; and genes that are involved in the plant responses to environmental stresses and signalling. It is also projected that a capability in bioinformatics to predict gene function "*in silico*" will be developed [IP5].

Genomics projects investigating the function of genes in apples and kiwifruit are currently underway at HortResearch. Using markers, genetic maps of these crops are being constructed. This kind of research has resulted in the creation of a variety of genetically modified organisms, including: bacteria to store the genes (gene libraries); transgenic bacteria and yeasts to express the protein products of the genes and determine the activity of the proteins; and transgenic plants that over-express or disrupt the expression of genes so that the function of the gene in the plant can be assessed ([IP5] Ross). HortResearch is also involved with proteomics, comparing protein data with the gene data to gain a better understanding of biological processes occurring in these crops.

Gene discovery may offer opportunities to improve crop and pasture plants. New Zealand Dairy Board is involved in sequencing ryegrass and white clover DNA [IP67] while AgResearch is actively developing sequence databases from these plants [IP13]. A key component of this work is the identification of genes expressed in different tissues at different stages of development and in response to various biotic and abiotic stresses.

New Zealand Forest Research Institute is also involved in the cloning and functional analysis of genes and with the modification of existing genetic traits in non-pathogenic microorganisms and plants such as *Arabidopsis thaliana* and *Nicotiana tabacum*, *Pinus radiata* and *Picea abies*. A number of transgenic *Pinus radiata* and *Picea abies* trees have been developed to evaluate the expression of

imported genes which include marker genes, those for antibiotic resistance, genes involved in herbicide resistance and genes involved in wood quality [IP2].

Marker assisted selection (MAS) is being used by HortResearch to develop new, non-transgenic varieties of crop in fast-breeding programmes. Fast breeding involves genetic modification in the laboratory during the development of markers, allowing identification of plants containing desired genes. These plants can then be bred and cross-bred traditionally. HortResearch has already employed these techniques on apples and kiwifruit and hopes to extend their use to develop bioremedial trees and shrubs that will accumulate toxic residues from soils ([IP5] Gardiner).

Another area of plant-related genetic research is into functional genomics of insecticidal microbes. Insect pathogens (microbes and nematodes that specifically kill insects) provide a rich source of insecticidal bioactive proteins and enzymes. Bioactive products can be identified using laboratory techniques such as gene disruption (eg transposons), cloning and sequencing of genes. For example, genes encoding insecticidal proteins have recently been discovered in a bacterial disease of grass grub. Genes with insecticidal function have the potential to be incorporated into other microbes or plant genomes as a means of achieving pest suppression ([IP13] Goldson).

Transformation in plant studies

To study the expression of particular genes during plant development, the genes are cloned in hosts (often in *E. coli*) to increase the number of copies of the gene. These genes are then isolated and used as probes to monitor the expression of the gene during development. In some cases the gene product can be made in the genetically modified plant and the proteins used for more study or the production of antibodies ([IP15] McManus).

New Zealand has been the site of field tests for genetically modified crops. These include ([IP4] Dunbier, Timmerman-Vaughan; [IP75], [IP14], [IP61]):

- canola and corn modified for herbicide tolerance
- sugarbeet modified for herbicide resistance or performance
- wheat modified to improve agronomic performance, to stabilise genetic variability or for disease and insect resistance
- potato varieties modified to be blight-resistant or potato cyst nematode-resistant
- barley varieties modified to improve performance or for disease resistance
- brassica varieties modified for virus resistance, club root resistance, aphid resistance or herbicide resistance

- onion modified to improve performance
- lentils modified for herbicide resistance
- asparagus modified for herbicide resistance or to delay post-harvest senescence
- broccoli modified to delay post-harvest senescence
- triticale modified to improve performance
- peas modified for resistance to alfalfa mosaic virus
- tamarillos modified for virus resistance
- various ornamentals modified to produce longer or stronger stems, or to have new flower forms.

While some of these transformed plants have been developed in New Zealand, others are imported for field testing.

In many instances, along with the genes of interest (perhaps from bacteria or other plants), selectable marker genes (for example, resistance to the antibiotic kanamycin) are also introduced into the plant genome.

White clover, an important pasture plant in New Zealand, has no known natural genetic resistance to some common pests such as grass grub and porina moth larvae. AgResearch and its collaborators have identified and isolated genes encoding insecticidal proteins (*Bacillus thuringiensis* d-endotoxins and several proteinase inhibitors from both plants and animals). The mode of action of the encoded protein has been determined, and the gene (*cry1Ba1*) transformed into white clover using an *Agrobacterium* vector. Before the transformation process, the gene was modified by truncating it and by making its sequence more closely resemble plant genes ([IP13] Woodfield).

Lincoln University has transformed two fungi that may have biocontrol properties. The fungi have been modified to contain a gene for antibiotic resistance so that they can be identified, and a gene which is expressed as an enzyme that produces a blue colour when provided with a particular substrate. This will allow study of the further growth of the fungi and how they parasitise their hosts ([IP8] Stewart).

AgResearch is involved in trying to increase the available energy in pasture plants by introducing genes controlling carbohydrates (particularly fructans) into perennial ryegrass and white clover. To this end, a number of genes controlling carbohydrate synthesis and partitioning have been isolated from bacteria, fungi and other plants, and their effects on plant carbohydrate levels are being investigated. Further work on the transforming of white clover and perennial ryegrass with fructan-synthesising genes has also started ([IP13] Woodfield).

Meanwhile Crop and Food Research ([IP4] Davies) has developed a capability for genetically modifying the biosynthesis of plant metabolites, especially flavonoids and carotenoids which are involved in flower and foliage colour. The research into flavonoids has involved the creation of new cultivars of ornamental crops. Flavonoids include a range of compounds which may be useful at reducing the rates of cancer and heart disease, and so further transformation work may involve food crops. Carotenoids are also involved in flower colour and have health-promoting compounds, for example the precursor to Vitamin A. Further research into gene transfer protocols may allow extension of this work from ornamental species to food crops.

Another area of investigation at Crop and Food Research involves locating genes implicated in post-harvest senescence and the control of the senescence processes [IP4]. This research will have implications in the shelf-life of food crops, as will investigations by HortResearch into the coordination of expression of stress-related genes ([IP5] Newcomb).

HortReseach is also starting research into plant development by studying "gene cascades", where expression of genes is controlled by other genes. It is also identifying genes that code for enzymes responsible for making flavour and "nutraceutical" compounds in fruit and is isolating these genes so that they may (in future) be manipulated in fruit species ([IP5] Newcomb).

AgResearch is involved in isolating promoter and terminator gene elements with defined expression patterns from forage plants for use in other programmes. This will allow the expression of transformed genes to be targeted to specific plant parts ([IP13] Woodfield).

Medical applications

Genetic modification techniques are widely applied around the world in medical research and diagnosis and in the production of treatments, the generation of specific immunoreagents and the generation of antibodies and drugs [IP37]. New Zealand is no exception. The work in New Zealand is being carried out in hospitals, universities and biotechnology companies. It involves the diagnosis and investigation of human disease conditions, including cancer, asthma, multiple sclerosis, autoimmune deficiencies, viral diseases, prenatal conditions, Duchenne and Becker muscular dystrophy, Fragile X syndrome, Huntingtons disease, haemophilia, spinocerebellar ataxias, multiple endocrine neoplasma and myotonic dystrophy ([IP59] Morris, Love).

Genetically modified mice are important in much medical research. They are predominantly used in New Zealand for basic biomedical research applications such as understanding pathological or developmental processes, modelling disease to aid the testing or development of new therapies, or in understanding gene function [IP45].

Diagnosis

Studies carried out in New Zealand include investigations into the molecular cause, progression, treatment and prevention of inherited or acquired diseases. Predictive disease testing, carrier testing, prenatal diagnosis and diagnostic confirmation of genetic disorders all rely on gene identification techniques.

Auckland Healthcare Services uses genetic modification techniques involving DNA hybridisation to investigate and diagnose genetic disorders. Genetic modification techniques are also used for the detection of carriers of genetic disorders (such as cystic fibrosis), predictive testing of individuals who risk developing a genetic disorder later in their lives, and testing individuals for predisposition to a disorder [IP91].

National Testing Centre also uses diagnostic genetic modification technology to screen newborn babies for metabolic diseases. Infants are screened for treatable disorders including phenylketonuria, maple syrup urine disease, congenital hypothyroidism, congenital adrenal hyperplasia, biotinidase deficiency, galactosemia and cystic fibrosis. Genetically modified products are also used in testing patients with symptoms for possible metabolic disorders [IP44].

Treatment

Many drugs available for the treatment of disease are the result of genetic modification technology. Some of these drugs and vaccines available in New Zealand ([IP59] Dixon) include:

- recombinant insulin for diabetes
- recombinant growth hormone to treat deficiency of this hormone
- erythropoietin for anaemia associated with renal failure or cancer
- recombinant human coagulation factors for haemophilia
- pulmyzyme for the treatment of cystic fibrosis
- cholera vaccine (live; subsequently withdrawn)
- monoclonal antibody for breast cancer
- plasminogen activator for myocardial infarction
- interferons α, β and γ

- interleukin-2 for cancer
- DNAase for cystic fibrosis
- α-1 antitrypsin for emphysema
- follicle stimulating hormone for infertility
- glucocerebrocidase for Gaucher disease.

Once diagnosed, some metabolic disorders, such as phenylketonuria (PKU), can be treated by diet modification. Others, such as Gaucher disease, may be treated by supplying the missing enzyme (glucocerebrocidase) which is produced by genetic modification technology. Much research is being done to develop other replacement enzymes and gene therapies [IP44].

In 1996, a gene therapy trial for Canavan disease was approved ([IP59] Dixon). The trial, which was not effective, was completed in 1997.

Cancer immunotherapy approaches using genetically modified mice have been used to establish and validate clinical trials where patients with non-Hodgkin's lymphoma are transfused with their own immune cells sensitised in vitro to recognise their own tumour cells as foreign [IP10].

Research

Genetically modified mice are particularly important in medical research. These mice may have genes inserted, mutated or deleted, often so that they mimic human diseases. Such studies in New Zealand ([IP45] Eccles, Hampton, McCormick) include:

- At Christchurch School of Medicine, a mouse model of the inherited disorder X-linked adrenoleukodystrophy has been developed and a mouse model lacking a cardiac hormone (BNP) is being developed.
- A team at AgResearch has developed a mouse lacking the STAT5b gene to study a model of a growth disorder.
- Researchers at Otago University use transgenic mice which express human genes involved with the development of heart disease. They are specifically interested in genes which are involved with the assembly of lipoproteins.
- Christchurch School of Medicine has imported mice with a gene knockout that prevents their white blood cells from making the chemicals necessary to kill bacteria. This genetic modification mimics a human condition known as chronic granulomatous disease (CGD).
- Mouse models lacking immune system genes are being developed by Malaghan Instutute to understand treatment of cancer, asthma and multiple sclerosis.

As well as genetically modified mice, research projects at Malaghan Institute use recombinant products including interleukins, interferons, colony-stimulating factors, peptide hormones and immuno-modulatory proteins.

Cancer research is a major investigative field in New Zealand. There are projects investigating the basis for, behaviour and development of novel treatments for cancers. This work is leading to the development of new approaches for cancer treatment, some of which have entered the clinical trial phase. Other work has led to the identification of the genetic cause for some types of tumour, for example stomach carcinoma. There are also investigations into why some tumours, such as breast cancers, spread, and how tumours have the ability to stimulate formation of new blood vessels [IP19].

Malaghan Institute has made investigations into how altered genes lead to a loss of control of normal cell development. This helps with understanding regulatory genes and their role in cancer. To better understand cytokine gene function, the Institute uses mice, modified either to not produce or to over-produce a cytokine, or mice which have modified immune systems [IP10]. Gene expression by cytokines is also being studied by University of Auckland to understand better the mechanisms involved in parturition in women. It is hoped that this will allow the development of treatments and strategies to prevent preterm births [IP27].

In other cancer research at Malaghan Institute, populations of tagged killer cells from genetically modified mice are monitored. Each population has a specificity for a unique tumour protein expressed by developing genetically modified tumours ([IP10] Harris).

University of Otago is also involved in cancer research and is using gene mapping to look at chromosome 14 deletions associated with renal cancer. Researchers are using genetic pedigrees and gene expression analysis to look at inherited susceptibility to gastric cancer in a large Maori family, and analysis of special gene mutations to identify pathways required for normal kidney development that are repressed in child cancer patients [IP27].

University of Auckland has a programme to examine the molecular basis of Huntingtons disease using transgenic mice and sheep models, and it is working towards developing gene therapy techniques for neurodegenerative diseases in humans [IP27].

Salivaricin B helps control streptococcal infections caused by *Streptococcus pyogenes*. This antibacterial protein, discovered by researchers at University of Otago, is produced by *Streptococcus salivarius* microorganisms. BLIS Technologies was formed to pursue the commercialisation of the Salivaricin B-producing *Streptococcus*

salivarius microorganisms, as well as the identification of other microorganisms producing bacteriocin-like inhibitory substances (BLIS) with human or animal health benefits ([IP26] Parker). Gene inactivation procedures are used to control the expression of these genes in bacterial hosts ([IP19] Tagg).

As part of its asthma studies, Malaghan Institute uses DNA polymorphisms in the beta2-adrenergic receptor to examine disease severity. Transgenic and knockout mice models are being used to examine the regulation of immune responses [IP27].

Genetic modification and genetically modified organisms are currently being used in New Zealand to better understand malignant hyperthermia (MH), a genetic syndrome, which usually has no symptoms unless the patient is exposed to certain types of anaesthesia ([IP15] Stowell).

University of Otago has identified mutations in a gene responsible for a rare disease in humans that causes blindness and kidney disease. In the laboratory, genetically modified bacteria, viruses, yeast, insect and mammalian tissue culture cells provide vectors and substrates for manipulation of DNA isolated from patients as part of investigations to identify specific features of the disease or experimental treatments for it [IP98].

Pure research and teaching

Some of the work described above may fit into the category of pure research, while some of the work described here could also fit into one of the above categories. Research and the application of that research are often intimately connected.

Teaching

New Zealand universities use genetic modification techniques, both for teaching and as research tools. Teaching involves not only lectures about the applications and results of genetic modification technology but also laboratory work on the fundamental techniques, including recombinant DNA technology [IP15].

Environmental effects of genetic modification

In addition to teaching, University of Canterbury engages in work with transgenic organisms to better understand horizontal gene transfer. This, in turn, aids understanding of the effects of release of genetically modified organisms into the environment [IP7]. AgResearch is also investigating horizontal gene transfer, particularly by bacterial plasmids in New Zealand soils ([IP13] Goldson]).

In related research, AgResearch scientists are looking at the environmental impact of transgenic plants developed to express insecticidal toxins. The research uses genetic manipulation involving cloning and expression of inserted marker genes.

This will enable the quantification of the effects of transgenic plants and breakdown products on soil ecosystems, including soil foodweb composition, biomass and nutrient status ([IP13] Goldson]).

HortResearch scientists, together with AgResearch, are analysing insecticidal transgenic plant impacts on bees, and investigating beneficial insects and soil microbes and nematodes ([IP13] Goldson]; [IP5] Malone).

Genomics

International work has mapped the human genome, and mapping of probes onto human chromosomes for gene identification has been done in this country. New Zealand has also been the site of work into the clarification of poorly mapped regions of the human genome ([IP59] Morris]).

Collaborative work between New Zealand and United States-based Physiome involves building a computer-based tool that will be used to create virtual cells, tissues and organs in a computer (biometrics). To run simulations, data regarding genes, proteins and protein interactions will be needed. This data will be generated using genetic modification technologies in laboratories world-wide ([IP23] Levin).

Brain process studies

Cellular mechanisms of learning and memory in the brain are being researched at Otago University. One of the aims of the research is to identify changes in gene expression in the brain during development of memory in a rat model. Genetic modification techniques will be used [IP27].

Food

Foods containing genetically modified ingredients are not commercially produced in New Zealand. However, various foodstuffs and feeds that are imported into New Zealand may contain ingredients which are genetically modified [IP56]. The main genetically modified ingredients are soybean, corn, canola, cotton, potato and sugarbeet. These are used in a wide variety of food products, including soups, sauces, processed meats, dairy products, baked goods, oils, spreads, confections and snack foods [IP54].

Funding of genetic modification research

Funding for projects that may include genetic modification technologies comes from both the government and the private sector and includes funding from local, national and international funding agencies and from charitable trusts and donations. Researchers report receiving funding from such government agencies

as the Health Research Council of New Zealand, Lottery Health Board, Marsden Fund, Public Good Science Fund and New Economy Research Fund, from charitable organisations and special-interest bodies such as the National Heart Foundation of New Zealand, New Zealand Dental Research Foundation, Cancer Society of New Zealand, Wellington Medical Research Foundation, Asthma Foundation, Multiple Sclerosis Foundation of New Zealand, Otago Community Trust, Otago Research Committee, and from international groups such as the Wellcome Trust and Novartis ([IP10]; [IP19] Tagg, Guilford).

The exact amount of money spent on genetic modification technology and research is unknown because budgets usually do not class genetic modification technologies separately from the broader research and development categories. However, some figures are available.

The recent Statistics New Zealand *Modern Biotechnology Activity in New Zealand* survey estimated that the enterprises they questioned spent around $405 million (in the year ending June 1999) on modern biotechnology; $276 million of this was the estimated expenditure for the private sector, while the public sector enterprises estimated that they spent $129 million. The value of income associated with modern biotechnology in the same year was estimated by the respondents to be $475 million. Private sector earnings were estimated to be $326 million of this figure, while the public sector earning were estimated to be $149 million. However, Statistics New Zealand urges caution in the use of the quantitative financial data, because of the difficulty experienced by the respondents in isolating modern biotechnological expenditure from their other expenditure.

FRST [IP21] invests about 80% of Government's overall research, science and technology funds. FRST estimates that $130–135 million may be invested in research programmes that may involve genetic modification technologies. Further, the Foundation estimates that genetic modification technologies are key in about 9% of projects in which it invests.

Health Research Council of New Zealand [IP27] is a government agency that was responsible for investing $40.3 million in health research in the 2000–2001 year. It estimated that 30% of the contracts it funded involved the use of genetic modification technology, accounting for about $16.1 million of the total.

AgResearch [IP13] estimates that it invests $25 million in research and development projects that involve genetic modification, genetically modified organisms and products. Crop and Food Research [IP4] estimates that its projects using molecular techniques as research tools received $2.5 million in funding, proof of concept projects that may or may not have used genetic modification received $3.1 million

and that $1.7 million of funding was spent on the development of genetically modified products. Landcare Research [IP12] estimates that it is currently involved in $2.8 million worth of genetic modification-related research, though less than $700,000 directly uses genetic modification or evaluates genetically modified products. New Zealand Dairy Board [IP67] has secured funding of up to $150 million for advanced biotechnology over the next five years.

Funding at Otago University for BLIS research (see above) over the period 1996–1999 has been estimated at over $1.5 million ([IP19] Tagg). University of Auckland Faculty of Medical and Health Sciences estimates expenditure of about $20 million annually on biotechnology-related projects: it estimates that Faculty of Engineering spends less than $500,000 annually on biotechnology and School of Biological Science around $13.5 million annually ([IP16] Condor).

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"Interested persons" referenced above (submissions and witness briefs are publicly available on the Commission website (http://www.gmcommission.govt.nz) until 30 June 2002):

[IP2] New Zealand Forest Research Institute

[IP3] Wrightson

[IP4] Crop and Food Research, including witness briefs from Dunbier, Timmerman-Vaughan, Davies

[IP5] HortResearch, including witness briefs from Gardiner, Malone, Newcomb, Ross

[IP7] University of Canterbury

[IP8] Lincoln University, including witness briefs from Bickerstaffe, Palmer, Stewart

[IP10] Malaghan Institute of Medical Research, including witness brief from Harris

[IP12] Landcare Research

[IP13] AgResearch, including witness briefs from Goldson, L'Huillier, McNatty, Woodfield

[IP14] Aventis CropScience

[IP15] Institute of Molecular BioSciences, Massey University, including witness briefs from McManus, Sarre, Stowell

[IP16] University of Auckland, including witness brief from Condor

[IP19] University of Otago, including witness briefs from Wallis, Tagg, Guilford

[IP21] Foundation for Research, Science and Technology

[IP23] Auckland UniServices, including witness brief from Levin

[IP25] Biotenz, including witness brief from Wakelin

[IP26] A2 Corporation, including witness brief from Parker

[IP27] Health Research Council of New Zealand

[IP28] New Zealand Veterinary Association, including witness brief from Squires

[IP34] Federated Farmers of New Zealand

[IP35] New Zealand Feed Manufacturers Association/Poultry Industry Association of New Zealand/Egg Producers Federation of New Zealand, including witness brief from Diprose

[IP37] Council of Medical Colleges in New Zealand

[IP44] National Testing Centre

[IP45] New Zealand Transgenic Animal Users, including witness briefs from Eccles, Hampton, McCormick, McLennan

[IP47] New Zealand Biotechnology Association

[IP54] New Zealand Grocery Marketers Association

[IP56] New Zealand Arable-Food Industry Council

[IP59] Human Genetics Society of Australasia, New Zealand Branch, including witness briefs from Morris, Love, Dixon

[IP61] Bio Dynamic Farming and Gardening Association in New Zealand

[IP67] New Zealand Dairy Board

[IP75] New Zealand Vegetable and Potato Growers' Federation/New Zealand Fruitgrowers' Federation/New Zealand Berryfruit Growers' Federation

[IP76] Environmental Risk Management Authority

[IP77a] Royal Society of New Zealand (biological sciences)

[IP85] SAFE (Save Animals From Exploitation)

[IP91] Auckland Healthcare Services

[IP92] New Zealand Association of Scientists, including witness briefs from Penny, Heath

[IP98] New Zealand Organisation for Rare Diseases.