MOLECULAR BIOTECHNOLOGY: APPLICATIONS IN LIVESTOCK SYSTEMS

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Summary

Molecular biotechnology in context of this section describes the application of molecular biology as a technology to improve livestock health and production. The DNA sequence of a number of livestock genomes has provided data which can underpin animal breeding programs to select breeds with desired traits (meat quality, milk production) and increased disease resistance. Similarly, livestock health can be improved through the advent of sensitive and specific diagnostic technologies and new generation vaccines through the availability of genomic sequence data. As biotechnology is applied to livestock, we can improve our approaches for managing breed traits and disease.

1. Introduction

Globally livestock production is growing faster than any other sector, and by 2020 the livestock sector is predicted to become the most important agricultural sector in terms of added value, referred to as the 'livestock revolution'. Agricultural biotechnology is a source of innovation in production and processing. Rapid advances in molecular biology provide new powerful tools for further innovation.

Animal diseases are a major and increasingly important factor reducing livestock productivity in developing countries in particular. The most promising applications of biotechnology to livestock systems is the improvement of animal health and production, in areas such as assisted reproduction, increased disease resistance, nano-based and refined diagnostic techniques, and increasingly improved vaccines with effective delivery systems. Use of DNA biotechnology in animal health may contribute significantly to improved animal disease control, thereby stimulating both food production and livestock trade.

2. Molecular biotechnology approaches applied to livestock systems

2.1. Genetics and breeding

Genetics is the science of heredity, the science attempts to explain the similarities and the differences that occur among related organisms. Nature is full of examples of traits that vary continuously eg. body size, reproductive ability, and if we examine many individuals in a population, we usually find significant differences among them. Often this type of variation can be quantified by measuring the trait in a sample of individuals from the population and the phenotype of every individual is reduced to a number. These numbers can be analysed with a variety of statistical techniques enabling us to study the trait and to investigate its genetic basis (thus 'quantitative traits'). Research in genetics has also revealed that the complex inheritance of quantitative traits is influenced by many genes and many factors in the environment.

Quantitative geneticists have developed techniques to identify individual genes that influence complex traits. Each gene occupies a specific position in the set of DNA molecules that constitutes the genome of an organism. These DNA molecules organized into discrete structures called chromosomes. A gene's position in a chromosome is call a locus (plural, loci), and the locus for a gene that influences a quantitative trait is called a quantitative trait locus (QTL). QTLs have been identified and mapped on specific chromosomes in model organisms such as the fruit fly and the mouse and also in livestock such as pigs (fatness and growth rates) and cows (milk production).

Driven by the commercial benefits of the cattle industry, cattle are by far the most studied domesticated animals. Initially cattle would have been bred for domestication for traits such as docility, size and the ability to survive stress. Later phenotypes such as breeding potential, early maturity, coat colour, horns and attributes associated with the production of commodities would have been exploited. This led to issues associated with inbreeding such as diseases, inherited disorders and reproductive problems. Crossbreeding was recognised as a way that some of these problems could be circumvented. Nevertheless, the long process of traditional breeding can now be aided by various assisted reproductive technologies (ARTs) combined with biotechnology approaches capable of increasing the rate of genetic progress.

2.1.1 Reproductive technologies

Traditionally, breeding data was used to identify inherited reproductive problems in domestic animals. Genes or mutations responsible for reproduction problems can now

be elucidated at the genetic level. Some ARTs applied to livestock to develop genetically valuable stock are:

1. Artificial insemination. AI is the first method developed and underpins the application of other ARTs applied to livestock. This procedure involves the collection of semen from males which is then used to impregnate females and has been applied in dairy cattle for over 65 years.

2. Multiple Ovulation and embryo transfer. MOET is the hormonal manipulation of females is undertaken to induce multiple ovulation prior to insemination which is then combined with the transfer of embryos into hormonally primed surrogates. This also allows offspring and milk to be produced outside usual seasons and has been shown to increase the rate of genetic improvement.

3. Transgenesis is the insertion of DNA into livestock and its stable integration into the germ line in order to rapidly introduce "new" genes into cattle, swine, sheep and goats without crossbreeding. This technique is important to many aspects of biomedical science including disease research, developmental biology, biomedicine, manufacturing and agriculture. Initially, genes responsible for growth have been exploited. The technology is currently very costly and inefficient and applications in the near future seem to be limited to the production of transgenic animals as bio-reactors.

4. Cloning is the production of offspring by embryo splitting (naturally or artificially induced to form 2 or more genetically identical animals) or nuclear transfer (creation of an animal from a reconstructed embryo made by transferring the nucleus of a donor cell into an oocyte from which the genetic material has been removed.

Of the above approaches AI and MOET are established in animal breeding. Transgenesis and cloning are not routinely used in domestic animal breeding programmes; due to costs, low efficiency and debated acceptance by consumers, politicians and scientists.

2.1.2 Molecular approaches to breeding

The ultimate goal of QTL mapping is to identify genes that underlie traits (meat and milk production, disease resistance) so as to better understand the physiological and biochemical functions associated with the trait. This then allows the development of marker assays which can be applied to screen animals involved in breeding programs to definitively breed for the desired inheritable trait. Mapping, which involves understanding the relative distances between genes on a chromosome, has been performed on many farm animals. Rapid progress has been seen in the last decade with movement from genome maps, to trait maps and to gene discovery.

Molecular genetics is the use of molecular markers to reveal polymorphisms at the DNA level and is increasingly applied in animal genetic studies – also termed 'marker-assisted' breeding or selection. Microsatellite DNA markers have been the most widely

used due to its easy application using PCR and electrophoresis. However, analyses using the detection of single nucleotide polymorphisms (SNP - change in one base within a DNA sequence) is also gaining popularity. This has led to development of commercially available DNA chips to screen populations of animals for thousands of markers (SNPs) in a single experiment. Resulting genotypes are incorporated into genetic association studies by identifying SNPs or markers in populations associated with complex phenotypic traits to assist in gene discovery. Another recent development is the concept of combining genome-wide expression profiling from microarray experiments with genetic linkage analysis. This assists to link the genotype with the phenotype and has been termed expression quantitative trait loci (eQTLs). There has been limited application to study livestock species to date with most studies targeting model species such as mice, however, the approach promises to greatly improve the understanding of the underlying causes of genetic variation.

Commercially available tests now available to breeders include tenderness (calpastatin gene), marbling (thyroglobulin gene) and fat deposition (leptin gene). The availability of the complete and validated genome architecture of chromosomes for livestock species is important for identifying the causative sequence variation underlying QTLs both within genes and in 'non-coding' areas of the genome which may play a role in gene regulation i.e. Callipyge trait in sheep. The chicken and cow genomes are now complete with pig and sheep planned for release in the near future.

2.2. Molecular diagnostics and animal health

Advanced biotechnology-based diagnostic tests make it possible to identify the disease-causing agent(s) at a species/sub-species/type level and to monitor the impact of disease control programmes. The appropriateness of a DNA or protein (antibody or antigen) based technique for a disease diagnostic method depends on the infectious agent (i.e. Prion – no DNA) and the type of the tissue the pathogen colonises.

2.2.1 Improved immunoassays/Immunosensors

Most conventional infectious agents elicit a host immune response; therefore diagnostic assays are developed to detect the specific antibodies produced in the host against the pathogen. Immunoassays take advantage of the affinity binding between antibodies (host) and the corresponding antigen (pathogen) allowing the detection of one of these in blood, serum and other biological fluids. Initially, radiolabeled immunoassays were developed which incorporated radioisotopes to monitor the distribution of free and bound antigen. Radioimmunoassays have mostly been replaced by the following approaches for antibody-antibody complex detection and measurement:

1. Fluorescence - antibodies linked to a fluorescent label, can be applied directly to examine tissue sections to microscopically directly detect antigens (pathogens) e.g. immunohistochemistry
2. Enzyme – using an antibody/enzyme conjugate producing a coloured reaction detectable using spectrophotometry called an enzyme linked immunosorbent assay (ELISA). This approach is also the basis of disposable tests (dipsticks) using membranes and colour reactions.

3. Chemiluminescence – emission of light to detect antibody-antigen aggregates
4. Light scattering – which measures the light scattering produced by antibody antigen aggregates
5. Electrochemical tags - which measure the redox potential by measuring current

There are 3 main types of immunoassay configurations:

1. Sandwich (immobilized antibody will bind to sample antigen which is detected by a labelled antibody),
2. Competitive (labelled antibody competes with the sample antibody to bind to an immobilized specific antigen), and,
3. Indirect (an immobilized antibody binds the sample antigen which is then bound to a primary antibody which is subsequently detected using a secondary labelled antibody).

New sensitive techniques are required to detect spongiform encephalopies (i.e. bovine spongiform encephalopy - mad cow disease). The prion's glycoprotein is very similar to that present in the host and only differs slightly in structure. Some developments include:

- Protein misfolding cyclic amplification (PMCA) which is conceptually analogous to polymerase chain reaction cycling but is able to generate the glycoprotein *in vitro* mimicking the aggregation which occurs in the brains of infected individuals
- Conformation-dependent immunoassay (CDI) which utilises recombinant antibody fragment directed against specific amino acids exposed in the prion but hidden the host protein. The assay is otherwise similar to a traditional ELISA. The protein sequence of the target and the ability to manipulate DNA in a modern biotechnology laboratory attributes to this ability to generate specific recombinant antibodies for the development of specific immunoassays.

While immunoassays describe tests based on immunoreactions, the term immunosensor is defined by a combination of an immobilised immunological receptor (antigen or antibody) and a transducer which converts a biological interaction into a measurable signal based on electrochemistry, fluorimetry, interferometry, resonance and reflectometry. Immunosensors have been developed for the diagnosis of bovine anaplasmosis and classical swine fever. Despite many of these new advances, the large proportion of tests are based on the more traditional immunoassays. Immunosensors offer the advantage of real-time monitoring with a large potential for veterinary application. Research is continuing to develop new surface chemistries, increase sample throughputs and to develop international standards.

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Biographical Sketch

Dr Lew-Tabor is currently a Senior Principal Research Scientist with Queensland's Primary Industries & Fisheries (QPI&F) based in Brisbane, Australia, and an adjunct Associate Professor for Murdoch University's Centre for Comparative Genomics in Western Australia. Since employed with DPI&F, she has been responsible for applying molecular technologies to solve animal health issues including: sensitive diagnostics, vaccine development, pathogen:host interactions and molecular epidemiology. Comparative genomics, phylogeny, sequence analysis and functional analyses have been the basis for the development of assays and treatments. She has published 65 peer reviewed publications and conference abstracts covering research into viral, bacterial, fungal and protozoal animal disease pathogens. She was a recipient of an Australian Academy of Science Europe exchange visit and collaborated with King's College London to study the molecular mechanisms of bovine host invasion by Babesia parasites linked to the UK group's research into malaria host invasion. Her molecular epidemiological techniques for tracing bovine anaplasmosis and babesiosis vaccination continue to be applied in cattle vaccination programs in Australia. She and her team have developed sensitive molecular diagnostic assays which have been subsequently adopted by Australian diagnostic laboratories and she has submitted protocols for Australia and New Zealand standardisation. Current research activities include the development of sensitive diagnostic techniques to detect bovine reproductive diseases (neosporosis, venereal campylobacteriosis, trichomoniasis, three day sickness) and screw worm fly (exotic to Australia); the molecular characterisation of strains of entomopathogenic fungi; development of a cattle tick vaccine (reverse vaccinology approach) and studies of the host mechanisms of tick resistance; and contributions to tick fever (babesiosis and anaplasmosis), cattle tick and venereal campylobacteriosis genome projects. Her current research programs include collaboration with a number of National and International research institutions and Universities.