

Part 3 Putting Technologies to Work

Crop Improvement

The applications of modern biotechnology to crops are in:

- Improved diagnosis of pests and diseases
- Tissue culture/micropropagation techniques
- The construction of transgenic plants with improved yields, disease, pest, and stress resistance, and/or nutritional quality
- The use of genetic markers, maps, and genomic information in marker-assisted and gene-assisted selection and breeding.

Diagnostics

The use of monoclonal antibodies and nucleic acid technologies has improved the specificity, sensitivity, and ease of diagnosis of plant pests and pathogens. These new diagnostics have also greatly assisted in the study of the ecology of pests and diseases, their more rapid identification in quarantine, and in the propagation of disease-free planting material.

Micropropagation Techniques

Tissue culture and other in vitro micropropagation technologies provide a practical means of providing disease-free plantlets of current varieties with significantly improved yield gains by the removal of pests and pathogens. Micropropagation,

when linked with new diagnostics, has been especially useful in vegetatively propagated crops such as sweet potato and banana and for the rapid propagation of tree species. Tissue culture is also a critical step in the construction of transgenic plants by enabling the regeneration of transformed cells containing a novel gene.

Modern Plant Breeding

The application of biotechnology to agriculturally important crop species has traditionally involved the use of *selective breeding* to bring about an exchange of genetic material between two parent plants to produce offspring having desired traits such as increased yields, disease resistance, and/or enhanced product quality. The exchange of genetic material through conventional breeding requires that the two plants being crossed be of the same, or closely related, species. Such active plant breeding has led to the development of superior plant varieties far more rapidly than would have occurred in the wild due to random mating.

Traditional methods of gene exchange, however, are limited to crosses between the same or closely related species. It can take considerable time to achieve desired results, and frequently, genes conveying desirable traits do not exist in any closely related species. Modern biotechnology, when applied to plant breeding, vastly increases the precision and reduces the time with which these changes in plant characteristics can be made, and greatly increases

the potential sources from which desirable traits can be obtained.

The application of recombinant DNA technology to facilitate genetic exchange in crops by transformation techniques has several features that complement traditional breeding methods. The exchange is far more precise because only a single specific gene that has been identified as providing a useful trait is being transferred to the recipient plant. There is no inclusion of ancillary, unwanted traits that need to be eliminated in subsequent generations, as often happens with traditional plant breeding. There has been some debate over the transfer of antibiotic marker genes with the single trait gene, and considerable research has now gone into eliminating the antibiotic marker genes from the final products prior to commercial use. Better still is to use markers that do not require antibiotics, such as new sugar-based markers.

The technical ability to transfer genes from any other plant or other organism into a chosen recipient means that the entire span of genetic capabilities available among all biological organisms has the potential to be genetically transferred or used in any other organism. This markedly expands the range of useful traits that ultimately can be applied to the development of new crop varieties.

The use of genetic markers, maps, and genomic information will improve both the accuracy and time to commercial use of single and polygenic traits in plant breeding (for example, the use of marker-aided selection in breeding for disease resistance in rice is illustrated in Box 5).

The present major technical limitation on the application of recombinant DNA technology to improving plants is insufficient understanding of exactly which genes control agriculturally important traits and

how they act to do so. This is the constraint that can be addressed through studies of plant genomes, as an aid to crop improvement.

The rapid progress being made in genomics should greatly assist conventional plant breeding, as more functions of genes are identified and able to be manipulated. This may enable more successful breeding for complex traits such as drought and salt tolerance. This would be of great benefit to those farming in marginal lands worldwide. Breeding for such complex traits has had limited success with conventional breeding in the major staple food crops.

Another important trait of great potential benefit to smallholder farmers would be apomixis. This is the ability to propagate plants asexually through seed. This would confer the benefits of hybrid vigor without the need to purchase new seed each season. Research is underway by scientists at CIMMYT, Mexico, working with other collaborators in France and the USA, to identify the genes conferring this trait.

Commercial Applications of Genetically Improved Crops

Substantial commercial cultivation of the first generation of new genetically improved plant varieties commenced in the mid-1990s. In 1999, approximately 40 million hectares of land were planted worldwide with transgenic varieties of over 20 plant species, the most commercially important being cotton, corn, soybean, and rapeseed (James 1999). These new crop varieties are planted in Argentina, Australia, Canada, China, France, Mexico, South Africa, Spain, and, predominantly, the United States. Approximately 15 percent of

Box 5 Molecular breeding: biotechnology at work for rice

Marker-assisted selection is the application of molecular landmarks—usually DNA markers near target genes—to assist the accumulation of desirable genes in plant varieties. There are many reasons why molecular markers are useful in plant breeding. Improved disease resistance in rice is a good example.

Bacterial blight is a widespread disease in irrigated rice-growing areas and can cause widespread yield loss. The incorporation of host-plant resistance through conventional breeding has been the most economical means of control, and has eliminated the need for pesticides. There are now over 20 genes available for use in rice improvement, but not all of these genes are equally effective in different environments. The pathogen eventually overcomes the resistant gene. Using conventional approaches the plant breeder must be continually adding and changing genes just to maintain the same level of resistance. Breeding effort spent in “maintenance” is a potential loss to gains in other traits.

A more sustainable system can be developed by deploying more than one resistance gene at a time. The challenge is to find the right combination of genes and put them into varieties most suitable for local production. When two or more genes are incorporated into a variety it is called “gene pyramiding.” Up to four genes for bacterial resistance have been pyramided in rice, and there is evidence that collectively they are more effective than would be ascribed to their additive effects. Because each gene may mask the presence of another gene, it is difficult to pyramid more than two genes by conventional breeding and selection; but it can be done with molecular markers.

Over the past several years, scientists at the International Rice Research Institute and its national partners in the Asian Rice Biotechnology Network have applied DNA marker technology to address the bacterial

blight problem. First, DNA markers are used to tag nearly all the bacterial blight resistance genes in available genetic stocks. Second, DNA markers are used to describe the composition of pathogen populations unique to each region. This parallel analysis of the host and the pathogen has enabled scientists to determine the right combination of genes to use in each locality.

In Asia, a number of resistance genes (Xa4, xa5, Xa7, xa13, Xa21), all with molecular tags, have been introduced in various combinations into locally adapted varieties.

The Asian Rice Biotechnology Network is promoting sharing of these elite lines and gene pyramids from different countries with other countries in Asia. This will allow the useful marker-assisted selection products to be rapidly disseminated through collaborative field testing across the region.

Marker-assisted selection has delivered some of the promises of biotechnology, and there are other examples of use in rice. The impact of new selection techniques will continue to be significant, particularly in an increasingly intellectual property-conscious environment. Marker technology is based on knowledge of endogenous DNA sequences; this has important practical implications, as the rice genome will be completely sequenced by an international effort, led by the Rice Genome Research Program of Tsukuba, Japan. As long as there is a public commitment to maintain all rice sequences in the public domain, useful genes for marker-assisted selection should be readily accessible to national and international rice breeding programs. Thus, because of their relative simplicity, easy integration into conventional breeding, and minimal background intellectual property, DNA marker technology and marker-assisted selection are expected to be strong driving forces in crop improvement in the future.

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the area is in emerging economies. The value of the global market in transgenic crops grew from US\$75 million in 1995 to US\$1.64 billion in 1998.

The traits these new varieties contain are most commonly insect resistance (cotton, maize), herbicide tolerance (soybean), delayed fruit ripening (tomato) and virus resistance (potato). The main benefits of these initial varieties are better weed and insect control, higher productivity, and more flexible crop management. These benefits accrue primarily to farmers and agribusinesses. There are also economic benefits accruing to consumers in terms of maintaining food production at low prices. Benefits also accrue to the environment through reduced use of pesticides, and the reduction in carcinogenic mycotoxins caused by fungal contamination in food crops.

Other crop/input trait combinations presently being field-tested include virus-resistant melon, papaya, potato, squash, tomato, and sweet pepper; insect-resistant rice, soybean, and tomato; disease-resistant potato; and delayed ripening chili pepper. Research is aimed at modifying the oil content (rape-seed), increasing the amount and quality of protein (maize), or increasing vitamin content (rice) (James and Krattiger 1999).

Much greater emphasis is now being given to improving the nutritional value of foods. There also is work in progress to use plants such as corn, potato, and banana as bio-factories for the production of vaccines and biodegradable plastics.

Characterizing Biodiversity

Genes and gene combinations selected in the past in nature and by humans will re-

main a vital source for germplasm improvement. They need to be conserved in seed banks, and in situ where possible and desirable. Genomics can play a key role in the characterization and conservation of genetic resources. It can be used to determine which genes and chromosome segments are duplicated, which are unique, and how easy it will be to recreate the various combinations of chromosome segments in modern plant breeding programs (Flavell 1998).

Comparative genetics can enhance exploitation of genebank collections. The CGIAR has an opportunity to become an important player in the field, by exploiting its own comparative advantages of germplasm management and enhancement and its international network of research centers and collaborators. The location of the Centers and their international network of collaborators coincide well with the centers of origin of the world's major food crops (see Map). Jointly, the CGIAR, national programs, and advanced laboratories now have an historic opportunity to work together to make optimal use of new developments in science, for the molecular characterization of agriculturally important species and their wild relatives amongst plant, livestock, and microbial genetic resources to achieve their goals.

The application of comparative studies to enhance the use and management of plant germplasm collections was the focus of an international workshop in The Hague in August 1999. It was co-organized by the International Rice Research Institute and the International Service for National Agricultural Research through the System-Wide Genetic Resources Program.

The major finding was that the CGIAR centers must take advantage now of the latest technologies in genomics research to apply comparative genetics to the germplasm collections that they hold in trust (System-Wide Genetic Resources Program 1999).

The principal conclusions from the workshop were that:

- Comparative genetics can provide the most precise, unambiguous and comprehensive tool for germplasm characterization
 - Cross-species comparisons will allow identification of the germplasm sources of superior, potentially optimal genetic sources for specific traits. Comparative genetics will provide a multilateral flow of knowledge between major and minor crops
 - CGIAR centers need to take the initiative to develop comparative genetics research for several crops, including cereals, roots/tubers, and legumes
 - Use of comparative genetics will help reposition CGIAR genebanks for the future and enhance use of germplasm in crop improvement programs
 - The strong comparative advantage of CGIAR centers to conserve, phenotype, and use germplasm should be linked with expertise in comparative genetics existing in many laboratories worldwide. This will require innovative investment and institutional arrangements
 - Additional investment by the CGIAR in comparative genetics and bioinformatics will ensure that the results and benefits are available as international public goods.
- The initial potential of comparative genetics may best be demonstrated with traits where gene action is simple and well understood, such as resistance to some pests and diseases, submergence tolerance, starch accumulation, nutritional qualities, phosphate uptake, resistance to soil toxicity, weed competitiveness, and flowering response. Comparative studies may facilitate:
 - The systematic search for useful genes that contain these traits in germplasm accessions without having to discover the genes for each crop
 - Identification of genetic resources containing useful genetic combinations
 - Understanding of the genetics underlying important traits
 - Better understanding of the structure of biodiversity that will enhance management of germplasm collections.

Comparative genetics provides the potential for trait extrapolation from a species where the genetic control is well understood, and for which there are molecular markers, to a species that has a limited amount of information. Rice, for example, is regarded as a model for cereal genomics because of its small genome. The similarity of cereal genomes means that the genetic and physical maps of rice can be used as reference points for the exploration of the much larger and more difficult genomes of the other major cereal crops, and be applied to the minor cereals. Conversely, decades of breeding work and molecular analysis of maize, wheat, and barley can now find direct application in rice improvement. These studies are much more advanced for cereals than for roots/tubers, and legumes. This reflects the large public

and private sector investments in the rice genome project, coordinated by Japan. This has recently been strengthened by the decision of Monsanto to donate its knowledge on the rice genome to the public sector effort. Other investments on the maize and wheat genomes in Europe and North America are making rapid progress.

The opportunities to apply comparative genetics now are furthest advanced in the cereals in which considerable research investment has already been made.

Investment in other agriculturally important species, especially for tropical crops such as cassava, banana, and food legumes, is limited. Without significant investment in the immediate future, the research gap between the CGIAR centers, national research institutes, and advanced laboratories already heavily involved in comparative genetics will widen. Collaboration with advanced laboratories is essential to exploit fully the potential of comparative genetics on all the agriculturally important species.

Bioinformatics

The CGIAR centers have gathered a huge resource of phenotypic data through the germplasm collections and the crop improvement and international testing programs conducted over the past 30 years. Research in molecular biology, genome sequencing, functional genomics, and comparative genetics are producing large amounts of new genomic data. Bioinformatics is essential for the management, integration, and analysis of phenotypic and genomic data if the promise of molecular biology for genetic improvement is to be realized.

New discoveries in comparative genetics indicate a high degree of conservation of genetic material across the genomes of many species. This applies in terms of gene order and gene structure and has important implications for the ability to translate findings in molecular biology in one species to others. This process will not be possible unless the bioinformatics tools are also compatible across species.

Numerous research projects worldwide are collecting genomic data. These are often made available for bioinformatic analysis in public databases. The task of linking these data resources, integrating the CGIAR's own contributions, and analyzing the products is too great for any one CGIAR center to handle. People with skill and experience in this new and rapidly changing field are rare and dispersed.

The CGIAR centers have a unique role to play in the design and deployment of a bioinformatics system for use by the international centers and their collaborators. CGIAR centers need to work together and with advanced research institutes and NARS partners to develop, deploy, and extend an integrated bioinformatics system for the major food crops. This will require new investments, new skills, and innovative organizational arrangements that cut across traditional commodity, discipline, and Center responsibilities.

Livestock Improvement

Constraints to Livestock Productivity

Three groups of technical constraints need to be overcome to improve livestock productivity in the developing world. These relate to improvements in genetic potential,

health, and management practices, including nutrition. In some cases these constraints are specific to tropical and subtropical environments, such as specific diseases and stresses. In others, the constraints are shared by industrial and developing countries.

Infectious diseases of livestock not present in the industrial countries, and for which there are as yet no sustainable means of control, present a formidable barrier to increasing the efficiency of livestock productivity in developing countries.

Disease is one of the major factors contributing to poor productivity of livestock in developing countries. In sub-Saharan Africa, animal losses due to disease are estimated to be US\$4 billion annually, approximately a quarter of the total value of livestock production.

Tsetse fly-transmitted trypanosomosis and tick-borne diseases are the most important disease problems in developing countries. Therapeutic agents are available for some of these diseases, but problems remain. Chemotherapy, based on the use of trypanocides, has problems due to toxicity, residues in milk and meat, and the excretion of large quantities in feces that are then applied to crops. Some of the trypanocides are potential carcinogens and would not be licensed for use in industrial countries. Intensive application is creating drug-resistant organisms.

Current drugs have been in use for over 30 years. The problem of drug resistance is becoming acute in some regions, and the likelihood that new drugs will be developed is low due to development costs and lack of return on investment. Vaccination offers a potentially more effective and sus-

tainable method of disease control (Morrison 1999), but technical challenges remain to be resolved.

There has been limited success in exploiting the genetic potential of indigenous livestock breeds to resist disease and environmental stresses and to better utilize the available natural feed resources. Further improvements in livestock genotypes now need to relate more to the quality of the final product and the efficiency of its production rather than simple increases in quantity. Improvements in animal health are moving from interventions at the level of the individual animal to interventions at the herd and flock health level, with a focus on prevention rather than treatment and subclinical rather than clinical disease. Vaccines play an important role in disease management by developing herd immunity to target diseases

Applications

The main applications of new biotechnologies to livestock are in the areas of genetic improvement, reproductive technologies, and animal health. These new technologies speed up the reproductive process in animals and enable the more efficient selection of breeds with improved productivity. Animal genome projects are also shortening the gene discovery process and demonstrating many potential applications where the manipulation of the genome may be useful in livestock improvement.

The fundamental differences in reproduction between plants and animals are reflected in the significant differences in the costs and efficiency of effecting production increases through breeding programs. These differences favor investments in crop rather than livestock breeding and

for short-term rather than long-term returns.

Phenotypes of commercial livestock breeds that are highly productive in temperate climates and intensive production systems do not realize their production potential in subtropical/tropical production systems. This is due to a number of factors including dietary constraints, adaptability to local environmental conditions, and susceptibility to disease.

National structures in developing countries, whether public or private, have often been unsuccessful in commercially exploiting the production capacities of indigenous livestock, which are adapted to the local environment and diseases, by selective breeding or some form of cross-breeding with exotic genotypes. This has been due to the need for long-term investment in such breed improvement programs and their complexity of management, especially when only small numbers of livestock are present on individual farms. Performance recording schemes are difficult to initiate and maintain, making breeding, selection, and expansion of improved livestock an expensive and inefficient process (Doyle 1993).

Molecular Breeding

Advances have been made in overcoming the genotypic constraints to increased production efficiency. Improvements have been made both in genetic characterization at the molecular level, and in technology to expand rapidly the available numbers of improved genotypes. In molecular characterization, linkage maps of sufficient resolution for use in breeding improvement schemes based on marker-assisted selection are now available for cattle, pigs, poultry, and fish. These maps are being refined, and the process of identi-

fying molecular markers with desirable biological and commercial traits is under way. The applications of these technologies to fish are illustrated in Box 6.

Another example of the use of molecular markers has been in tracing the origins of different cattle breeds. Genomes contain the history of the origin and evolution of the different cattle breeds and modern molecular techniques have been used to rapidly decipher their story (Bradley and others 1996; Hanotte and others 2000).

The physical location of individual genes on chromosome maps is also well advanced. The rapid development of both linkage and physical maps of the genomes of domestic livestock is a clear example of how the large investment in basic biology (the construction of genetic maps of mouse and humans) can effectively and economically be captured to the benefit of domestic livestock improvement.

The International Livestock Research Institute (and previously the International Laboratory for Research on Animal Diseases) has been involved for the past decade in a worldwide collaborative effort to create and improve the genetic maps of the bovine genome and to identify markers associated with genetic resistance to trypanosomiasis. The use of such maps will significantly reduce the generation time for developing improved breeds, as compared to conventional breeding procedures based solely on phenotype selection. The determination of genetic distances, together with genetic maps, also will increase the effectiveness of measures for conservation of endangered livestock species by allowing characterization at the genetic rather than phenotypic level.

The application of comparative genomics between breeds and species may mean that such selection strategies in one

Box 6 Applications of biotechnology in fisheries and aquaculture

There is growing importance of molecular markers for biodiversity research, genome mapping, and trait selection in fish and other aquatic organisms. International groups are already collaborating on developing genetic maps of tilapia, common carp, salmonids, catfish, zebrafish, and pufferfish. Maps for commercially important invertebrate species including shrimp and oysters are being initiated.

The feasibility of developing and using transgenic species of fish is being explored by several research institutes and companies in the fisheries and aquaculture sectors on various species including tilapia and salmon. It is anticipated that there will be an increase in the number of species and strains into which genes are introgressed, and the number of gene constructs available for transgenesis (governing biological functions in addition to growth) will also be increased. Transgenesis may become a cost-effective means of enhancing indigenous species important to one or a few countries and not covered by international breeding efforts.

Sex manipulation (for example the production of all male populations of fish, especially tilapia) is also an active area of research, designed to avoid the detrimental production effects of early maturation and cessation of growth. In carp species, however, all-female populations are required. It is also anticipated that sex reversal will be used more widely in breeding programs to increase the speed of production of inbred lines. Haploid fish will be important for similar reasons.

A wide range of new molecular diagnostic techniques is being developed for applications such as disease diagnosis, sexing of juvenile fish, and for assessing progeny relationships in large populations of fish raised together to reduce environment-specific variations in production. Other techniques include tissue culture, or other manipulations of embryos or embryonic cells, for the isolation of viruses, bacteria, and fungi pathogenic to fish.

Source: International Center for Living Aquatic Resources Management.

species/breed may be more easily adapted to that of other species/breeds, when looking for similar traits. However, because of the high cost, genomics technology is presently being applied more to the lucrative markets, breeds, species, and production environments of the industrial world than to the needs of livestock improvement in the developing countries.

The concerted application of modern breeding strategies for livestock of relevance to smallholder production in developing countries is unlikely to occur in the absence of major public sector initiatives.

This is because of present lack of funding, the low commercial value of the breeds, lack of effective conventional breeding programs in developing countries, and the requirements to conduct selection in the relevant production environments due to high genotype x environment effects in animal breeding.

The applications of biotechnology to fisheries and aquaculture offer the prospect of increasing the efficiency of protein production, and the speed of conversion of feed to protein. They may also enable economically efficient protein production in enclosed aquaculture, and reduce problems of effluent disposal (see Box 6 for applications).

Transgenic Livestock

Technology exists for the creation of transgenic livestock including mammals, birds, and fish. Practical applications of the technology are presently restricted to production of human biological pharmaceuticals in the milk of sheep. Small herds of transgenic animals are likely to be able to produce sufficient quantities of high-value biological products, such as pharmaceuticals, in the immediate future. There has also been work on the creation of transgenic lines of virus-resistant poultry, which contain a modified virus gene that confers disease resistance. A similar strategy has proved useful to introduce virus resistance in plants.

In the future, transgenic pigs may be used as a source of tissue and organ transplants to humans, provided safety and ethical concerns are met. The major health issue for review and research at present is the possible trans-species movement of viruses from pig tissue to humans.

The impact of transgenic animals on animal breeding and production is presently limited by the dearth of single gene traits in livestock, and the fact that the propagation process of a transgene in an animal population is relatively slow (Cunningham 1999). There is also a risk that the desired gene may not be inherited by subsequent generations or may be turned off in the offspring from a transgenic animal.

If desired genes controlling a trait can be identified and transferred, their expression physiologically controlled and the trait is heritable, then a quantum leap in improvements in livestock productivity can be envisaged.

Livestock Genetic Resources

There is potential to find genes for disease tolerance and other adaptive traits such as heat tolerance in wildlife and transferring these to domestic livestock. In disease resistance, for example, this would have greater impact in developing than in industrial countries.

There are possible opportunities for the developing countries, through the analysis of the genomes of their unique animal genetic resources, to identify genes encoding traits that may be of benefit to both developing and industrial countries. Although the animal genetic resources in developing countries are plentiful, they have not been tapped to any great extent. The characterization of these genetic resources may offer opportunities for developing countries to benefit from their appropriate use and agreed benefit sharing (FAO 1999).

Diagnostics and Therapeutics

Molecular technologies are also applicable to the study of livestock parasites and other pathogens. They provide effective means for identifying, isolating, characterizing, and producing molecules that can be used to induce protective responses against the parasite (Morrison 1999). The new technologies can also be used to generate products and gene sequences, which can form the basis of improved diagnostics. They also provide effective means of elucidating the metabolic pathways of pathogens that confer drug resistance or drug sensitivity on these organisms. Genetic markers are increasingly being used to identify with

greater precision the species, subspecies, and types of pathogenic agents. Recombinant or genetically modified pathogens also offer new approaches to vaccine delivery, as does direct injection of DNA into animals.

Disease, however, is the result of the interactions of two genomes - the pathogen and the host. To exploit the new technologies, particularly for the development of vaccines and for the exploitation of disease resistance traits, it is important to understand the biology of the pathogen as well as the host.

Vaccine Development

The use of vaccines in disease control has the advantage of using an already existing domestic animal gene pool. From a management/breeding point of view this is logistically easier to consider as a disease control strategy than using marker- or gene-assisted selection to breed disease-resistant strains of improved domestic animals. Vaccines developed using traditional approaches have had a major impact on the control of the epidemic viral diseases of livestock, such as foot-and-mouth disease and rinderpest. There are many other important diseases, notably parasitic diseases, for which vaccines have not been developed successfully.

Rapid advances in biotechnology and immunology over the last two decades have created new opportunities to develop vaccines for parasitic diseases. Initial optimism in the early 1980s that vaccine products would quickly emerge from applications of recombinant DNA technology has not been fully realized. Subsequent experience has demonstrated that, unlike traditional approaches to vaccine development, effective

exploitation of recombinant DNA technology requires knowledge of the target pathogens and the immune responses they induce, as well as an understanding of how those immune responses can be manipulated. Such information was lacking in the early 1980s. There has been a series of fundamental discoveries in immunology that have led to a detailed understanding of how the immune system processes and recognizes pathogenic organisms, and the different ways that infections are controlled by immune responses. This new knowledge is directly relevant to all stages of vaccine development, from identification of the genes or proteins that need to be incorporated into a vaccine, to the design of a vaccine delivery system that will induce a particular type of immune response. These advances, coupled with further developments in the application of DNA technology, now provide a strong conceptual framework for the rational development of new vaccines (Morrison 1999).

Two main approaches are being pursued to develop vaccines using recombinant DNA technology. The first of these involves the deletion of genes that are known to determine virulence of the pathogen, thus producing attenuated organisms (nonpathogens) that can be used as a live vaccine. This strategy is presently more appropriate for viral and bacterial diseases than for protozoan parasites. Attenuated live vaccines have been developed for the herpes viruses that cause pseudorabies in pigs and infectious bovine rhinotracheitis in cattle.

The second strategy is to identify protein subunits of pathogens that can stimulate immunity. This is the preferred approach to many of the more complex pathogens. It requires knowledge of the

immune responses that mediate immunity, which helps identify the relevant target proteins. The strategy can be illustrated by the approach taken by the International Livestock Research Institute to develop a vaccine against *Theileria parva*, the parasite that causes East Coast Fever of cattle in sub-Saharan Africa. Studies of immune responses to the parasite have revealed antibody responses to the tick-derived infective stage of the parasite, as well as cell-mediated immune responses against the parasite stages that reside within cattle cells. A parasite protein recognized by the antibody response and the corresponding parasite gene (p67) have been identified. Protein expressed from this gene, when used to vaccinate cattle under experimental conditions, has been shown to protect a proportion of animals against the disease. Identification of the parasite proteins recognized by the cell-mediated immune responses presents a greater challenge, but a number of recently developed methodologies for this purpose are now being applied to the problem (McKeever and Morrison 1998). As part of its strategy for the development of a vaccine against East Coast Fever, the International Livestock Research Institute is collaborating with The Institute for Genomic Research to map the genome of *T. parva* (Nene and others 2000) (see Box 4).

New Vaccine Delivery Systems

Live attenuated vaccines stimulate immune responses similar to those induced by the parent pathogen, and usually provide long-lasting immunity. Vaccines using killed organisms require incorporation of adjuvants (agents that enhance immunity-giving characteristics), and the immune

responses they induce are usually more limited and of shorter duration than with live vaccines. Advances in biotechnology have provided a number of alternative vaccine delivery systems for subunit proteins that overcome these shortcomings and offer some of the advantages provided by live vaccines. Two of the most promising approaches are the use of attenuated organisms as live vectors and vaccination with DNA (Morrison 1999).

Live-vectored vaccines involve the incorporation of a gene encoding a subunit protein into the genome of an attenuated organism, which itself may be in use as an attenuated vaccine. The protein is then produced when the organism replicates in the animal.

A vaccine containing a rabies virus gene has been used to protect foxes against rabies and its use has resulted in the eradication of rabies from northern continental Europe.

The use of DNA for vaccination is based on the discovery that injection of genes in the form of plasmid DNA can stimulate immune responses to the respective gene products. This occurs as a result of the genes being taken up and expressed by cells in the animal following injection. Stimulation of immune responses and partial protection have been reported for a number of pathogen genes in livestock species, but none of these has yet led to a fully effective vaccine. The live vector and DNA vaccination systems are amenable to further manipulation to enhance the immunity-conferring characteristics of the gene products. Experimental studies have demonstrated that these systems have enor-

mous potential for development of vaccines that induce appropriate and enduring immune responses.

New vaccines are likely to be produced against some or all of the major animal diseases, given the necessary scientific and financial resources. The complexity of the problems that are being addressed should not be underestimated. The opportunities presented by advances in biotechnology can only be exploited effectively if there is a thorough understanding of the biology of the target pathogens and the diseases they produce. The new technologies allow detailed studies on the two interacting genomes, the pathogen and the host, the

identification of genes essential for causing infection and disease and thus the identification of targets for vaccine development.

Vaccine development for domestic livestock could benefit from technology spillovers from vaccine development for humans because the same research concepts and approaches can be applied, albeit to different pathogens. Public-private sector cooperation emerging in the eradication of polio, and in the search for a malaria vaccine, may yield innovative research cooperation models and knowledge that could be applied to vaccine development and delivery for the benefit of smallholder livestock producers in the developing world.