The genetic engineering of production traits in domestic animals

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Abstract. The transfer of recombinant DNA by microinjection of embryo pronuclei provides a novel approach to the manipulation of production traits in domestic animals. In this review, several of the key areas currently under investigation are examined and their progress evaluated. These include the manipulation of the endocrine system by altered growth hormone genes and the modification of animal biochemistry by the introduction of the cysteine biosynthetic pathway and the glyoxylate cycle. The possibilities inherent in the alteration of structural proteins important to domestic animal productivity, and some ethical issues relevant to the release of modified animals are also considered. The experimental information obtained so far in the area indicates that transcriptional regulation of the genes and a thorough understanding of the physiological processes involved are both important factors in the practical application of the technique to the improvement of animal productivity.
Key words. Genetic engineering; domestic animal productivity; growth hormone; wool growth; cysteine biosynthesis; glyoxylate cycle.

Introduction

The productivity of domestic animals is a function of their genetic potential and their interaction with the environment. Environmental factors are transient and are only partially subject to influence by the farmer. For example, farmers have significant control over animal health and supplementary nutrition, but have very little influence over important factors such as rainfall, temperature and environmental pollution. On the other hand, genetic potential is a much more stable property, and concentrating on its improvement is important if substantial cumulative gains in productivity are to be made. Historically, the genetic potential associated with important animal production traits such as wool growth, milk yield and body growth has been improved by selective breeding, whereby phenotypically superior animals are used as parental stock for following generations. The approach has been highly successful, as witnessed by the high quality of the domestic animals in use in farming today compared with those of earlier centuries. Nevertheless, the method has several significant limitations that have frustrated animal breeders for many years. First, the progress in genetic gain is slow, averaging, at best, about 1%–3% per year. This results from the complex genetic interactions that combine to produce a particular animal phenotype. Secondly, it is often difficult or impossible to separate a desired production trait from one or more undesirable traits. Thirdly, and perhaps most importantly, it is not possible to transfer genetic information between species, because of the biological barrier that prevents inter-species breeding. This precludes the transfer of advantageous genes from one species to another.

All the above difficulties arise from the mechanism of transfer of DNA between animals, whereby whole chromosomes constitute the unit of genetic exchange. The recent revolution in molecular genetic techniques now offers a possible solution to some of the above breeding difficulties, although it should not be considered a panacea. The successful transfer of recombinant DNA to mouse embryos by the technique of pronuclear microinjection of single-cell embryos[9, 16, 25, 49, 52] and the subsequent demonstration that the technique could be used to alter the growth properties of mice[26, 27], heralded one of the most significant technical advances in modern biology. When taken together with current sophisticated methods for the isolation, characterisation and manipulation of specific DNA sequences, the technology provides the ability to alter the genetic properties of animals without recourse to conventional breeding. Furthermore, small pieces of genetic information can now be transferred in isolation, instead of the complex assortment of genes involved in whole chromosome exchange. Application of the methodology to agriculturally important animals is commercially attractive, and laboratories around the world have committed significant resources towards the adaptation of the laboratory mouse techniques to larger animals. The first transgenic pigs and sheep were reported in 1985[18], and in the 5 years since that report, further sheep[23, 38, 40, 47] and pigs[18, 31, 32, 51, 53] have been reported, together with initial reports on the production of transgenic cattle[44, 45], fish (see Houdebine/Chourrout, this multi-author review) and chickens (see Shuman, this multi-author review). The range of transgenic animals produced indicates that most, if not all, major domestic animal species can be genetically modified by the technology.

While gene transfer overcomes many of the difficulties associated with conventional breeding techniques, its application is, nevertheless, not a simple matter. The exquisite precision with which genetic modification of an animal can now be achieved brings with it a new set of problems. Foremost amongst these is the need to understand the physiological processes of domestic animals at a molecular level. This is necessary to enable genes that

influence important production traits to be identified and isolated. It has become apparent over the past few years that very few such traits actually fall into this category. Secondly, it becomes important for the appropriate molecular modifications to be made to the chosen gene so that its effect on the animal is as desired. Thirdly, it is important to determine whether, in the process of gene insertion, an existing gene of importance has been damaged, which will require a detailed assessment of the general physiology of the animal.

Production traits amenable for manipulation in domestic animals

Genetic engineering can alter the factors that control the growth and body composition of domestic animals or the products that derive from the animals. In theory it is easier to manipulate animal products than the actual body composition of animals, because product manipulation would be expected to have a lower impact on the animal's general physiology. Nevertheless, most attempts at animal manipulation have involved growth and composition rather than products, presumably reflecting the paucity of genetic knowledge concerning the latter. The various areas amenable for manipulation are:

1. The endocrine system
2. Biochemical pathways
3. The structural proteins of the textile fibres and milk
4. The immune system

In the following pages, the attempts to manipulate the endocrine system, biochemical pathways and structural proteins will be reviewed. Disease resistance and the immune system are covered separately (see Müller and Brem, this multi-author review). Finally, some general conclusions will be drawn concerning the probable commercial application of the technology in the next decade and beyond.

The modification of the endocrine system of domestic animals

Since 1985 a considerable effort has been put into attempts to manipulate the endocrine status of domestic animals by genetic engineering and thereby modify their productivity. The results have been reviewed in detail several times in recent years [17, 31, 41, 56–58] and so in this section the overall conclusions from the work will be summarised. The general approach emanates from the pioneering research of Palmiter, Brinster and their colleagues who demonstrated that by altering the concentration of circulating growth hormone in transgenic mice the growth rate and final size of the animals could be increased [26, 27]. Their results were achieved by the use of genes containing a promoter sequence from the mouse metallothionein-I (MT-I) gene and the coding sequence for the rat or human growth hormone polypeptide. Circulating growth hormone levels were constitutively elevated in the transgenic animals, and increased skeletal growth was obtained. The experiments were then repeated in pigs, sheep and rabbits [18], using the same MT-I promoter and either the human or the bovine growth hormone coding sequence. Expression of the transgenes was first obtained in transgenic pigs [17, 31, 32] and constitutive elevation of growth hormone levels was obtained. In contrast to the mice, however, the transgenic pigs did not show elevated growth, despite being more efficient in feed utilisation. Moreover, the animals were physiologically abnormal [31, 32]. When similar results were obtained with transgenic sheep, it became clear that the larger domestic animals were not responding to the elevated growth hormone concentrations in the same way as laboratory mice [38, 39, 41].

The initial results in pigs and sheep were obtained with transgenes encoding heterologous growth hormone proteins (bovine or human), and the possibility was raised that the poor growth response was due to poor recognition of the new hormone molecules. Some support for this explanation was provided by a report of a faster-growing transgenic pig which contained a transgene encoding porcine growth hormone [51]. However, such an explanation now appears unlikely, because transgenic sheep with elevated levels of the natural sheep growth hormone exhibited the same poor growth and abnormal physiology as did those sheep with excess heterologous hormone [23, 24]. A more likely explanation for the poor growth in the larger animal species is that they become acromegalic. Rexroad et al. [39] showed that transgenic sheep with excess bovine growth hormone were diabetic, a typical symptom of acromegaly, and ascribed at least a part of their poor growth performance to this condition. These animals also contained a greatly elevated concentration of the growth factor IGF-1. Nancarrow et al. [24, 58] conducted a detailed analysis of the physiology of transgenic sheep containing extremely high levels of the natural sheep growth hormone, and arrived at similar conclusions to those of Rexroad and his colleagues. The animals had an elevated basal metabolic rate and an associated high cardiac output, IGF-1 was elevated, and renal function was impaired. Bone growth was abnormal, particularly in the front limbs, and the internal organs of the transgenic sheep were significantly larger than controls. The animals showed obvious symptoms of diabetes and all died within one year of birth.

From the above experiments, it is clear that chronic high production of hormone must be avoided for the practical application of the research. Different approaches have been taken to achieve such a goal, but as yet none has been successful. Pursel et al. [31, 32] have produced animals containing modified genes encoding the growth hormone releasing factor. These animals produced elevated levels of the releasing factor, but the concentration of growth hormone itself was not altered. As a result, the transgenic animals remained healthy and of normal appearance, and did not grow at any faster rate than controls. Wagner [53] has attempted a different approach by retaining

the coding sequence for the growth hormone, but placing it under the control of a promoter sequence isolated from the gene encoding the enzyme phosphoenolpyruvate carboxykinase. The expression of this transgene can be regulated by the ratio of carbohydrate: protein in the diet, but again no growth of the transgenic animals beyond that of controls was obtained.

The modification of the biochemistry of domestic animals

Genetic engineering is certain to have a large impact on animal production through the introduction of genes that alter the inherent biochemical capability of animals. The evolutionary process has resulted in the loss of numerous genes for biochemical pathways that produce compounds important for various production processes. These must be supplied as essential nutrients in the diet. Genetic engineering now provides the ability to transfer genes encoding these pathways from organisms where the pathways are functional. In order for this to be useful, however, it is necessary to identify substrate or nutrient limitations to important production traits. This is not a simple task, because many production traits are influenced by a variety of different factors depending on the actual husbandry situation, and the identification of these factors requires a detailed knowledge of the physiology of the production trait. Such knowledge is often not available.

The cysteine biosynthetic pathway

The Australian wool industry provides one useful area where this approach might succeed. Reis et al.[34–37] showed that when sheep are fed a diet that simulates that of the grazing animal, the amino acid cysteine is rate-limiting for wool growth. While cysteine itself is not an essential amino acid for mammals, it can only be synthesised from methionine. Thus the supply of either methionine or cysteine is essential in the sheep diet. In order to increase the rate of wool growth, it is clearly necessary to increase the supply of one of these two amino acids. However, direct dietary supplementation is not effective because the added amino acid is degraded in the rumen of the sheep by the resident microflora. Since this problem was identified, several different approaches have been taken to overcome the degradation that occurs. In one approach, proteins which have a low ruminal degradability are included in the diet. These proteins can be of natural origin, or can be 'protected' by treatment with formaldehyde[13], which prevents ruminal degradation but allows digestion in the distal part of the digestive tract. Alternatively, methionine itself can be encapsulated in a medium that survives the rumen but not the lower digestive tract[13]. While these methods have all proven effective in increasing the amount of available cysteine, they have not been economical in the practical farm environment.

An alternative approach is to introduce into the sheep genome the genes that encode the cysteine biosynthetic pathway. These genes are functional in prokaryotes and the auxotrophic eukaryotes, and are thus available for isolation, modification for expression in the digestive tract of sheep, and transfer to the sheep genome by embryo microinjection. Two laboratories are currently pursuing this approach in Australia, using the genes from *Escherichia coli*[54,55,57] or *Salmonella typhimurium*[11,43,48]. The current status of the research can be summarized as follows.

The pathway for the biosynthesis of cysteine in *E. coli* and *S. typhimurium* is complex and consists of two discrete parts, namely, a pathway for the reduction of a sulphur source to sulphide, and a simple two-step pathway that combines the sulphide with the amino acid serine to produce cysteine. The two genes in *E. coli* that encode the second part of the process are the *cysE* and *cysK* genes, encoding the enzymes serine transacetylase and O-acetylserine sulfhydrylase. These catalyse the following reaction:

$$\text{serine} + \text{acetyl-CoA} \xrightarrow[\text{serine transacetylase}]{} \text{O-acetylserine} + \text{CoA-SH}$$

$$\text{O-acetylserine} + H_2S \xrightarrow[\text{O-acetylserine sulfhydrylase}]{} \text{cysteine} + \text{acetate}$$

The *cysE* and *cysK* genes from *E. coli* and the *cysE, cysK* and *cysM* genes from *S. typhimurium* have all been isolated and fully characterised[3,11,12], thus providing the necessary coding sequences for the gene transfer experiments.

The research involved in this type of genetic engineering project can be divided into four parts:

1. Isolation and characterisation of the appropriate DNA coding and promoter sequences
2. Construction and testing of various fusion genes in cell culture to determine the optimum configuration of the various components
3. Testing of the preferred gene in transgenic mice to determine the efficiency and tissue-specificity of expression in vivo
4. Transfer of the preferred gene to transgenic sheep

The modification of the genes for expression in eukaryotic cells has been achieved in different ways. In our own laboratory, the bacterial coding sequence has been inserted downstream from the sheep metallothionein-Ia promoter with the sheep growth hormone gene used for stabilisation of the mRNA transcribed from the fusion gene product[54,57]. The general design of these genes is shown in figure 1. Their ability to be transcribed and translated in eukaryotic cells was tested in mouse L-cells. The genes all produced mRNA transcripts of the predicted sizes[54], and extracts prepared from the transformed cells contained readily detectable levels of the enzymes SAT and/or OAS (table 1). However, the importance of investigating a number of different combinations of the various components used to construct the fusion genes is shown by the substantial differences in

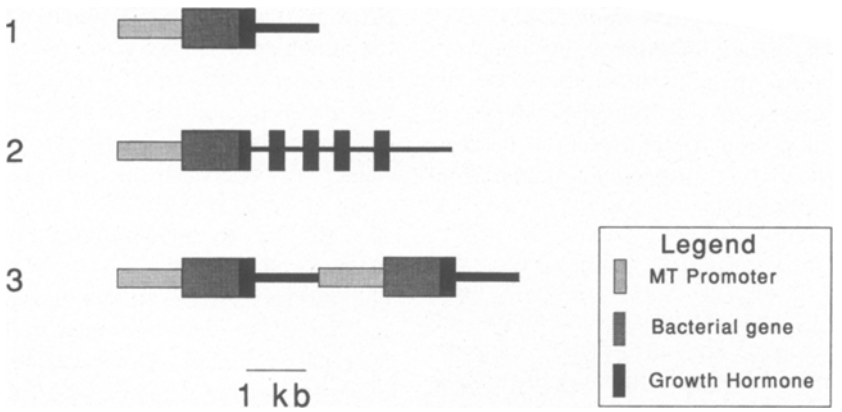

Figure 1. Diagrammatic representation of the modifications made to bacterial coding sequences to provide expression in eukaryotic cells. Gene 1 contains exon 5 of the sheep growth hormone gene 3′ to the bacterial sequence. Gene 2 is similar but contains the entire sheep growth hormone gene. Gene 3 consists of a fusion of two gene 1 sequences such that a single piece of DNA encodes the enzymes necessary for the cysteine synthesis or glyoxylate cycle biochemical pathways.

expression observed with the different genes. Genes containing only exon 5 of the sheep growth hormone gene (MTCE10, MTCK7 and MTCEK1) were expressed at much higher levels than genes containing the entire growth hormone sequence.

Several of the cys-encoding fusion genes have been transferred to transgenic mice, where their expression has been measured in intestinal, liver and kidney tissues. Initially, sequences encoding either *cysE* or *cysK* were examined, and these results showed that only those genes containing exon 5 of the sheep growth hormone gene located 3′ to the bacterial coding sequence were expressed at detectable levels in zinc-induced transgenic mice. This information is of general relevance to the expression of genes in transgenic animals, since the genes containing only exon 5 do not possess any exon/intron structure. Introns therefore are not an obligatory requirement for the expression of foreign genes in transgenic mice.

Having established the fact that both genes can be independently transcribed and translated in transgenic mice, the transfer of a combination gene which contains both the *cysE* and *cysK* sequences in a single piece of DNA (fig. 1) was examined. The transcription and translation of both *E. coli*-derived sequences have been demonstrated in these animals upon zinc induction (table 2), thus establishing them as unique mammals possessing the enzymes necessary for the utilisation of H_2S. The expression of the *cysE* and *cysK* genes was found in the intestinal epithelium, and the *cysK* gene was also expressed in the kidney and liver. Expression was dependent on induction by zinc. Clearly, the actual biosynthesis of cysteine in these animals requires the presence of the necessary substrates in the tissues which express the transgenes, and current experiments are directed towards establishing whether such biosynthetic activity can be demonstrated in the mice.

The results obtained from transgenic mice indicate that a combination of genes appears to have been established that is suitable for expression in animals, since high levels of zinc-inducible expression of both genes has been obtained in the intestinal epithelium. Earlier studies in our laboratory have shown that there is close correspondence between the expression in transgenic mice and transgenic sheep, although the zinc status of sheep can result in genes that are regulated by metallothionein promoters

Table 1. Activity of serine transacetylase (SAT) and O-acetylserine sulfhydrylase in extracts from cells transformed with various fusion genes containing the *cysE* (CE) and/or *cysK* (CK) genes of *Escherichia coli*. Genes were constructed as shown in fig. 1. pMTCE10, pMTCK7 and pMTCEK1 contain only exon 5 of the sheep growth hormone gene. pMTCE11 and pMTCK11 contain the complete sheep growth hormone gene. Enzyme activity is expressed as nmoles substrate utilised (SAT) or product formed (OAS) /30 min /mg protein.

Construct	Zn-induced		Uninduced	
	SAT	OAS	SAT	OAS
pMTCE10	2796	–	777	–
pMTCE11	255	–	68	–
pMTCK7	–	1350	–	–
pMTCK11	–	162	–	–
pMTCEK1	268	6960	86	1242

Table 2. Activity of serine transacetylase (SAT) and O-acetylserine sulfhydrylase (OAS) in tissue extracts prepared from transgenic mice. CK7-26 contains the gene pMTCK7, CE10-29 contains pMTCE10 and EK1-28 contains pMTCEK1. Specific activity is measured as nmoles substrate utilised (SAT) or product formed (OAS) /30 min /mg protein.

Mouse line	Organ	SAT	OAS
CK7-26	Intestine	–	206
	Kidney	–	352
	Liver	–	13
CE10-29	Intestine	6546	–
	Kidney	0	–
	Liver	0	–
EK1-28	Intestine	15144	519
	Kidney	0	938
	Liver	0	156
	Brain	0	90

being more highly expressed in sheep than in mice[23,46]. We have therefore commenced the transfer of these genes to sheep.

A slightly different approach has been taken by Rogers et al.[11,43,48] to achieve the same goal of enabling sheep to synthesise cysteine from H_2S. The source of genes to provide the appropriate coding sequences is again a bacterium, in this case *S. typhimurium*. The two genes being used are the *cysE* gene, which is essentially the same as that of the *E. coli* gene, and the *cysM* gene, which encodes an OAS enzyme significantly different from that encoded by the *cysK* gene both in *E. coli* and *S. typhimurium*. These two bacterial genes have been expressed in sheep cells in culture after being fused to the SV40 late promoter and SV40 polyadenylation signal sequences[43,48], where they have given rise to active SAT and OAS enzyme activities. More recently, the two genes have been joined together in a single piece of DNA and each coding sequence placed under the control of a promoter derived from the long terminal repeat of the Rous sarcoma virus. When the whole construct was transferred to mice by pronuclear microinjection, constitutive expression was obtained[48]. The same gene has also been transferred to a number of transgenic sheep and constitutive expression of SAT and OAS observed in tail tissue[43]. These experiments are significant because they demonstrate that the transcriptional and translational products of the genes, in spite of their bacterial origins, are stable in at least some sheep tissues. The research now in progress aims to provide the genes with a promoter more suited to directing their expression in tissues where the appropriate substrates for cysteine biosynthesis might be available[43].

The glyoxylate cycle

The introduction of a cysteine biosynthetic pathway to sheep to improve wool growth is the most advanced of the research projects designed to modify the biochemistry of domestic animals in order to improve productivity. However, another project that is well advanced in our own laboratory is designed to improve the general efficiency of feed utilisation in sheep, and to specifically increase nutrient supply to sheep wool follicles. This research involves the introduction of the glyoxylate cycle to sheep.

The digestive process in ruminant animals is largely dependent on the bacteria, protozoa and fungi which populate the rumen. These microorganisms consume essentially all available carbohydrate in the ingested feed and produce a range of fermentation products, the most important of which, from an energy viewpoint, are the volatile fatty acids. These are absorbed by the sheep and are used directly for energy or, if gluconeogenic, are converted to glucose to provide the carbohydrate that is essential for the proper function of several key tissues. Included in these tissues are the wool follicles, which have a high demand for glucose[6].

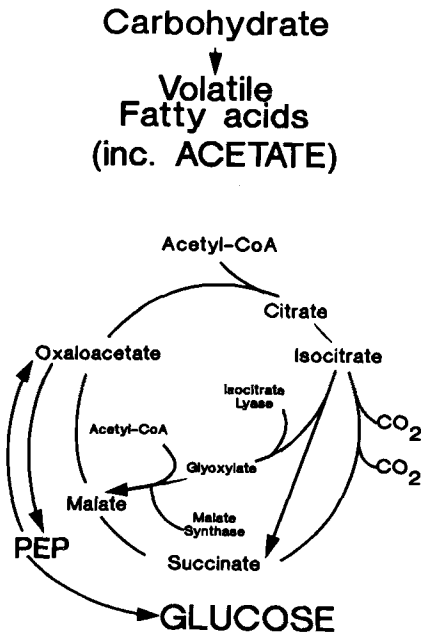

Figure 2. The biochemical reactions of the glyoxylate cycle. PEP = phosphoenolpyruvate.

On some pastures, the predominant volatile fatty acid produced in the rumen is acetate, which is not gluconeogenic. Sheep on such pastures are prone to ketonuria and a sub-optimal growth of wool is predicted[1,19,50]. It has been suggested that if the abundant supply of acetate in these animals could be utilised for glucose production, some of these problems might be overcome.

Acetate can serve as a source of glucose in organisms that possess the enzymes necessary to catalyse the reactions of the glyoxylate cycle[7]. The glyoxylate cycle is shown in figure 2. Acetate enters the tricarboxylic acid cycle in normal fashion to produce isocitric acid, but when the glyoxylate cycle is operational, the isocitrate is cleaved to succinate and glyoxylate by the action of the enzyme isocitrate lyase. Succinate is a gluconeogenic substrate, whereas the glyoxylate produced in the reaction is combined with another molecule of acetate to produce malate, thus providing the necessary substrate for continuation of the cycle. This second reaction is catalysed by the enzyme malate synthase.

In *E. coli*, the enzyme isocitrate lyase is encoded by the gene *aceA*, while malate synthase is encoded by the *aceB* gene. These have both recently been isolated and sequenced[4,21,42]. In order to transfer this genetic information in functional form to sheep, a protocol similar to that described earlier for the cysteine biosynthesis genes is being followed. The general construction of the genes is shown in figure 1, utilising the sheep MT-Ia promoter and exon 5 of the sheep growth hormone gene in the same way as described above for the cysteine biosynthetic genes. Three genes have been tested for expression in mouse L-cells and transgenic mice.

Table 3. Activity of isocitrate lyase and malate synthase in extracts of zinc-induced L-cells transformed with fusion genes encoding the enzymes of the glyoxylate cycle. pMTaceA1 and pMTaceB1 contain the complete sheep growth hormone sequence, while pMTaceA2 and pMTaceB2 contain only exon 5, as shown in fig. 1. Specific activities are expressed as nmoles of product formed /20 min /mg protein and are corrected for a low level of malate synthase activity in L-cell control extracts. No isocitrate lyase activity was detected in untransformed L-cells.

Construct	Isocitrate lyase	Malate synthase
pMTaceA1	76	–
pMTaceB1	–	1.7
pMTaceA2	68	–
pMTaceB2	–	34.3

When transferred to L-cells in culture, all three genes produced RNA transcripts of the predicted sizes[2, 57] and these were translated into active enzyme as indicated by the activities of isocitrate lyase and malate synthase in extracts prepared from the transformed cells (table 3). The genes were then transferred to mice and their expression examined in liver, kidney and intestinal tissues. The genes produced mRNAs in these tissues which hybridised with appropriate probes for the bacterial coding sequences and were of the predicted sizes[2]. Cell-free extracts from the same tissues showed active isocitrate lyase and malate synthase activities, indicating that the animals have the enzymic potential for the operation of the cycle[2]. The detailed physiology of these animals is currently under investigation.

Other possible biochemical pathways

The general concept of biochemical pathway manipulation for improved domestic animal productivity is assuming wider acceptance as the full potential of gene transfer becomes apparent. Thus it has recently been suggested that the *E. coli* genes for threonine biosynthesis and lysine biosynthesis could be used to introduce the biosynthetic pathways for both amino acids into transgenic livestock[33]. This paper included a computer simulation study of the metabolite flux that might ensue if the appropriate enzymes were to be produced in animals. However, their proposal also highlights some of the technical difficulties inherent in the methodology at present. Chief amongst these are:

a) The need for control of expression of the introduced genes

Experiments in which the levels of circulating growth hormone have been manipulated in transgenic animals have shown that uncontrolled release of growth hormone in pigs and sheep leads to severe physiological disorders[23, 24, 31, 32, 58]. Regulation of the transgene in these experiments is mandatory. However, the requirement for control is likely to be more general. For example, the research directed towards the manipulation of biochemical pathways generally requires the use of pre-existing cellular substrates and co-enzymes (e.g. the cysteine biosynthetic pathway, which uses acetyl-CoA and serine, or the glyoxylate cycle, which utilises isocitric acid and acetate, and synthesises succinate and malate). While there may be situations where the constitutive expression of the transgenes in these experiments is acceptable, regulation of expression is in general a desirable goal. However, there is a dearth of suitable promoters for such regulation of transgenes, and research to provide such control elements should have high priority.

b) The need for pathways with as few steps as possible

When experiments are aimed at introducing new metabolic pathways to animals, it is desirable to encode the entire pathway on a single piece of DNA. However, the technical difficulties associated with the manipulation of large pieces of DNA places a limit on the number of genes that can be considered. The lysine biosynthetic pathway, for example, is probably beyond the capability of current technology. A minimum of 8 genes would be required, and if a configuration similar to that used to modify the *E. coli* cysteine biosynthesis genes were to be used, the size of the DNA would exceed that which can be readily manipulated in common cloning vectors. For example, each gene would require about 1 kb of promoter sequence, 1–2 kb of coding sequence, about 0.5 kb of 3′ sequence for polyadenylation signals, and a minimum of 1 kb of spacer DNA to separate the different genes. This amounts to at least 27–28 kb of DNA, which would constitute a formidable cloning and construction task and would also probably be very difficult to transfer to animals by microinjection of embryo pronuclei. While inserting the complete pathway as a mixture of smaller pieces of DNA is a technical possibility, the problems this would create in the analysis of the transgenic animals and the difficulties associated with subsequent breeding of progeny make such an approach unattractive. Thus, until alternative methods for the transfer of genes to domestic animals are perfected (e.g. the use of embryonic stem cells), the limit for a pathway would seem to be 4–5 genes, provided that the coding region was of the normal bacterial size of about 1–2 kb.

Structural protein modification

Structural proteins are a useful target for genetic engineering because their modification would not be expected to affect the viability of the animal. The potential for such modification remains speculative at present, for very little experimentation is currently directed towards this goal. However, the growing acceptance of the relevance of transgenic animals in domestic animal production has resulted in several approaches in this area receiving serious consideration. The most obvious proteins of commercial value are: 1. the proteins of the textile fibres (wool, cashmere and mohair); 2. the proteins of milk.

The scope for modification of the textile fibres

Animal hair, which includes the important textile fibres wool, cashmere and angora, is composed of a unique group of sulphur-rich proteins, the keratins[14]. These

proteins combine in a precise arrangement of microfibrils and surrounding matrix, the geometry of which is essentially the same in all fibres. The different properties of the various fibres arise partly from differences in keratin protein composition and partly from differences in cell structure and arrangement that arise during fibre production in the follicle. The exact contribution of each of these factors to the final properties of the fibre is uncertain, but it is probable that manipulation of unique keratins may alter the textile properties of the fibre. Changing specific keratin proteins by gene modification requires a detailed knowledge of the genes involved. This information is still incomplete, but nevertheless is now sufficient for some experiments to be considered in this area.

The keratins can be divided into sub-groups, each of which has an important role in the final epidermal structure. Each sub-group of keratins is encoded in the genome by a multi-gene family, and gene probes for each of these families have now been isolated[59]. Many of the genes encoding the wool keratins have been isolated[15, 28, 30, 60] and their sequences are being examined for conserved regions that might indicate potential control and regulatory regions. Recently, however, an important experiment was reported in which a complete keratin gene encoding a type II wool protein was expressed in transgenic mice[29]. The gene was expressed at different levels in the various transgenic animals, and when this expression was high, the wool protein caused significant abnormalities in the structure of the mouse hair. This is probably the result of incompatible pairing of the wool protein with the mouse keratins and hence provides some guide to the limits for keratin manipulation. The importance of the experiment lies in its demonstration that a complete keratin gene can be expressed tissue-specifically in another species, thus opening the way for the minor modification of such genes and observing the effects of the modifications on wool structure.

We have already seen that wool biosynthesis is limited by the supply of the sulphur amino acids because of the high cysteine content of the wool keratin proteins. An alternative approach to increasing the supply of cysteine, already described, is to reduce the cysteine requirement of the fibre. This might be achieved by modifying one or more of the genes encoding matrix keratins so that the number of cysteine residues is reduced, and then transferring the modified genes to animals such that they are expressed at high levels. Since the cysteine content of wool can vary considerably without apparent effect on its textile properties (P. J. Reis, unpublished), the expression of low-cysteine matrix keratins could increase wool production substantially, and experiments to test this hypothesis are currently in planning.

The desirable textile qualities associated with cashmere fibre arise partly from the fine diameter of the fibre (<16 μm) and partly from a cuticle structure different from that of wool[20]. The factors that are responsible for the structure of the cashmere cuticle are not known, but good candidates are the cuticle-specific proteins that fill the cells. Recently, several genomic sequences encoding cuticle-specific proteins have been identified[22]. This opens the way for comparison of the wool protein and the cashmere protein. If significant differences are observed, the cashmere protein could be transferred to sheep, where its expression in the wool follicle might alter the wool fibre cuticle to a cashmere-like cuticle. This would be more straightforward than breeding for 'two-coatedness', which has also been advocated (J. Lax, personal communication).

Other modifications to the structural proteins of the textile fibres are possible. For example, proteins with increased numbers of amino acids possessing side-chains important for dye-retention might be introduced into the fibre. Inclusion of proteins with specific toxicity to moths might prevent the destructive action of the keratinases secreted by these insects. It may also be possible to reduce or eliminate the shrinkage that wool fabrics can undergo during normal washing. These examples remain speculation at this time, but serve to demonstrate the ever-widening range of possibilities that is opening up in the area of textile fibre protein modification.

Milk proteins

The mammary gland is an attractive target for genetic engineering because, like fibre production, it also offers the possibility of genetic manipulation without affecting the viability of the engineered animal. There are two ways in which milk production can be altered. Firstly, new genes can be designed which encode foreign proteins of high value, e.g., pharmaceuticals and growth factors. This concept, which uses the transgenic animal as a bioreactor, has been the subject of considerable research over the past few years (see Wilmut et al., this multi-author review). It is the subject of a separate chapter and will not be covered further in the present review. The second way in which milk production can be modified involves alteration of the genes encoding existing milk proteins.

Milk is a stable micellar suspension of protein, lipid and inorganic ions. The protein component is complex, consisting of a family of caseins which stabilise the milk micelles, and a water-soluble albumin-like component external to the micelles. Considerable variation can occur in the protein: lipid ratio of milk, with present consumer preference tending towards higher values. Thus, manipulation of the genes encoding the milk proteins to favour higher protein content would be of significant advantage, provided that the protein content was maintained at a level compatible with the general micellar structure of milk.

One approach would be to increase the expression of one or more of the casein genes by altering the promoter sequences that control their expression in the mammary gland. This could be achieved either by introducing addi-

tional enhancer elements into the existing promoter, or by replacing it with a promoter from a more highly expressed mammary-specific gene. While this is simple in concept, however, it may prove to be difficult in practice. Alterations to the beta-lactoglobulin gene by introduction of additional coding sequence significantly down-regulated the level of expression of the gene in transgenic sheep[8], suggesting that expression of this gene is not wholly a function of 5′ sequences, and recent evidence indicates that expression of the casein genes is influenced by sequences other than those immediately 5′ to the coding regions (A. G. MacKinlay and K. A. Ward, unpublished).

Another approach would be the partial inhibition of lipid biosynthesis in the mammary gland. The inhibition of the transcription of an existing gene is more difficult than adding a new gene activity, but two techniques have now shown some promise in mammalian systems. The first is the use of anti-sense RNA, which has effectively reduced the transcription of genes in transgenic animals. The second technique, still of very recent invention, involves anti-sense RNA in combination with the self-catalytic RNA cleavage structure known as the ribozyme. The latter technique has proven more effective than conventional anti-sense RNA in inhibiting gene transcription in mammalian cells in culture, but has yet to be demonstrated in transgenic animals. The application of either of these techniques could be used to inhibit the production of one of the key enzymes involved in lipid biosynthesis. Another milk protein modification of potential value is the reduction in concentration of alpha-lactalbumin, which is deleterious to cheese production. This could also be achieved by the use of anti-sense RNA or catalytic anti-sense RNA.

Community acceptance of genetically-engineered animals

While the introduction of transgenic animals into the farming community is probably some years away, some genetically-engineered livestock will eventually be used by farmers to improve productivity. There exist at present wide-spread community concerns about the safety and long-term utility of such animals, and it is a responsibility of the scientists involved to initiate the processes that are necessary to allay these fears. This can only be achieved by a combination of community discussion and effective legislation, organised in such a way that the science necessary for the development and testing of the animals is not impeded but providing at the same time a safe and open regulatory framework for release of the animals.

Several countries, including Australia, have addressed the question of legislation required to fulfil the above goals. A wide range of regulatory regimes currently operates in different countries, from a very liberal approach in Italy to extremely strict requirements in Germany and Denmark. Canada, the UK, the US and Australia all use similar methods for the review and controlled release of genetically-engineered organisms, and in the Australian situation, a Government enquiry has been underway since mid-1990 to formalise the procedures that must be followed in this country. The difficulties associated with the production of suitable legislation become clear when one considers the various interest groups involved in the release of genetically-engineered animals. Thus, the general public need an assurance that their health and environment are adequately protected, interest groups need a mechanism for expressing their concerns, scientists need clear guidance on the requirements for release so that they can design appropriate experiments and politicians must take into consideration the economic needs of the nation and yet at the same time properly represent the concerns of their local constituents.

One possible approach to this problem would be to involve the major public interest groups in discussions of genetic engineering research projects at an early stage in their development. In this way, many of the environmental concerns of the public could be addressed and allayed well in advance of the time when release of the modified animals is required. The objection to this approach is the loss of commercial confidentiality. However, there are many projects that could be discussed in general terms without breaching the confidentiality needed for the establishment of patents to protect the techniques, genes and animal strains involved. (It is recognised here, but not as a view accepted by the authors, that people can object to the patenting of livestock on general ethical grounds).

It is important that the question of community acceptance be addressed as a matter of priority, because it is likely that the public perception of genetic engineering may become as great a hurdle to the practical application of the research as the actual production of useful animals.

The future direction for genetic engineering of domestic animals

The technology of genetic engineering is still in rapid flux, making it difficult to predict future developments with any degree of certainty. Nevertheless, it is clear that the approach works at the laboratory level. Making the transition to significant improvements in productivity is an even more difficult problem, however, because the delicate physiological balance of the animals is easily disrupted leading to lowered overall performance. The work on the manipulation of animal biochemistry that has been described here will serve as a useful guide to the extent to which animal metabolism can be altered in general. In the next few years, there is likely to be more emphasis placed on the production of animals with altered structural protein production. This will include animals which produce foreign compounds in their milk, and the approach may also be extended to encompass the production of compounds in other secretions of the ani-

mal, either for commercial production of novel compounds or for use by the animal.

Another area of major research during the next five years is likely to be the alteration of the activity of existing genes. Powerful new approaches to this technology are now available, including the use of genes encoding antisense RNA molecules and RNA molecules that contain catalytic cleavage sites (ribozymes)[5,10] which can attack and destroy specific mRNA molecules. In addition, the replacement of existing genes with modified sequences may soon be possible in domestic animals. This involves the use of embryonic stem (ES) cells, the production of which remains in its infancy in domestic animal research.

In the euphoria accompanying the initial discovery and implementation of the techniques of genetic engineering, many promises were made and potential benefits described to the farming community. In the light of subsequent experience, it is apparent that productivity gains will be hard-won and will require the combined expertise of molecular geneticists and physiologists. The farming community has been waiting patiently for the promised transgenic animals and the onus of proof is now firmly with the scientists. However, the research yet required is substantial, long-term and costly, and it is to be hoped that the farmer's patience will be sufficiently durable.

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