Chapter 1
Transgenic Farm Animals

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Introduction

Conventional science to improve muscle and meat parameters has involved breeding strategies, such as selection of dominant traits or selection of preferred traits by cross breeding, and the use of endogenous and exogenous hormones. Improvements in the quality of food products that enter the market have largely been the result of postharvest intervention strategies. Biotechnology is a more extreme scientific method that offers the potential to improve the quality, yield, and safety of food products by direct genetic manipulation. In the December 13, 2007 issue of the Southeast Farm Press, an article by Roy Roberson pointed out that biotechnology is driving most segments of U.S. farm growth. He indicated that nationwide, the agriculture industry is booming and much of that growth is the result of biotechnology advancements. For example, the United States produces over half the worldwide acreage of bio-engineered crops (GMO), and this growth is expected to continue worldwide. With respect to livestock, biotechnology is a more novel approach to the original methods of genetic selection and crossbreeding, or administration and manipulation of various hormones (i.e., growth).

Biotechnology in animals is primarily achieved by cloning, transgenesis, or transgenesis followed by cloning. Animal cloning is a method used to produce genetically identical copies of a selected animal (i.e., one which possesses high breeding value), while transgenesis is the process of altering an animal's genome by introducing (via gene transfer) a new or foreign gene (i.e., DNA) not found in the recipient species, or deleting or modifying an endogenous gene with the ultimate goal of producing an animal expressing a beneficial function or a superior attribute (e.g., adding a gene that promotes increased muscle growth). The gene or genes that are transferred or modified is called the transgene (TG). A combination of the two methods, i.e., transgenic cloning, is the process of producing a clone whose

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donor cells contain heritable DNA inserted by a molecular biology technique, as used in a transgenic event. The first to report on creating cloned animals was Hans Dreisch in the late 1800s. Dreisch's intent, however, was not to create identical animals but rather to prove that genetic material is not lost during cell division. His research experiments involved sea urchins, which he intentionally chose, since sea urchins have large embryo cells and grow independently of their mothers. A pioneering report by Palmiter et al. (1982) on the accelerated growth of transgenic mice that developed from eggs microinjected with a growth hormone (GH) fusion gene started the revolution in biotechnology of animals. Based on this research, many novel uses for biotechnology in animals were envisioned, beginning with the enhancement of production-related traits (yield and composition) and expanding into disease-resistance strategies and production of biological products (i.e., pharmaceuticals). The primary goal of transgenesis is to establish a new genetic line of animals, in which the trait is stably transmitted to succeeding generations. The past several years involving transgenic research has primarily focused on altering carcass composition, increasing milk production, enhancing disease resistance, and reducing excretion of phosphate by pigs. A significant amount of progress has been achieved. However, the success of this research is dependent upon improving the efficiency of the nuclear transfer technology, which will in turn reduce the cost of producing transgenic animals.

Early methods of cloning involved a technology called embryo splitting, but the traits of the resulting clone were unpredictable. Today's method of cloning, i.e., somatic (adult) cell nuclear transfer, became established in 1996 with the production of the world's first cloned farm animal, "Dolly" the sheep (Wilmut, Schnieke, McWhir, Kind, & Campbell, 1997), at the Roslin Institute in Scotland, and has since been used for cattle, goats, mice, and pigs. Cloning could be a promising method of restoring endangered, or nearly extinct, species and populations. Production of transgenic animals is carried out by a technique called pronuclear microinjection, reported first in mice (Gordon, Scangos, Plotkin, Barbosa, & Ruddle, 1980), and later adapted to rabbits, sheep, and pigs (Hammer et al., 1985). An excellent review on genome modification techniques and applications was published by Wells 2000).

Before 1980, applications for patents on living organisms were denied by the U.S. Patent and Trademark Office (USPTO), because anything found in nature was considered non-patentable subject matter. However, the U.S. scientist Amanda Chakrabarty, who wanted to obtain a patent for a genetically engineered bacterium that consumes oil spills, challenged the USPTO in a case that landed in the U.S. Supreme Court, which in 1980 ruled that patents could be awarded on anything that was human-made. Since then, some 436 transgenic or bio-engineered animals have been patented, including 362 mice, 26 rats, 19 rabbits, 17 sheep, 24 pigs, 20 cows, 2 chickens, and 3 dogs (Kittredge, 2005). Due to the steps specific to transgenic procedures, for instance the DNA construct, its insertion site, and the subsequent expression of the gene construct, animals derived from transgenesis have more potential risks than cloned animals. Based on a National Academy of Sciences, National Research Council (NRC) 2002 report, "Animal Biotechnology:

Science-Based Concerns," the U.S. FDA in 2003 announced that meat or dairy products from cloned animals are likely to be safe to eat, but to date has not yet approved these products for human consumption. More recently (2007 and 2008), the U.S. FDA has reported that meat and meat from cloned animals is as safe as those from their counterparts bred the old-fashioned way. However, progress in this area is very slow and has a long way to go before having an impact at a commercial usage level. It still will be years before many foods from cloned or transgenic animals reach the shelves in stores, mainly for economic reasons. At an estimated cost of $10,000–$20,000 for each bio-engineered animal, these technologically engineered animals are a lot more expensive than their ordinary bred counterpart. Thus, producers will be more inclined to use the bio-engineered offspring for meat and not the cloned or transgenic animal itself. The U.S. Department of Agriculture (USDA), however, recommended that the U.S. farmers should keep their cloned animals out of the market place indefinitely, even as FDA officials claim that food from cloned livestock is safe to eat.

Bio-engineered foods are regulated by three agencies: USDA, Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). The USDA has an oversight for meat and poultry, whereas seafood regulation falls under the FDA. The FDA Center for Veterinary Medicine (CVM) also regulates transgenic animals because any drug or biological material created through transgenesis is considered a drug and will have to undergo the same scrutiny to demonstrate safety and effectiveness (Lewis, 2001). The EPA has a responsibility for pesticides that are genetically engineered into plants. In the mid-1980s, federal policy declared that biotechnologically derived products would be evaluated under the same laws and regulatory authorities used to review comparable products produced without biotechnology. As stated on the FDA website, the CVM has asked companies not to introduce animal clones, their progeny, or their food products into the human or animal food supply until there is sufficient scientific information available on the direct evaluation of safety.

Characterization of Candidate Genes/Genetic Markers for Carcass and Meat Quality Traits

Animals vary widely in their genetic merit and commercial value. Classical selection techniques have been utilized, over the years, with great success for improving animal production traits, but the underlying genetic changes were elusive to researchers in the past. Technological advances in molecular biology in the early 1990s opened up a whole new area of investigations into the DNA genome. Presently, there is a lot of attention being paid to the identification and sequencing of chromosomal regions representing quantitative trait loci (QTL) influencing carcass traits, growth, and meat quality factors. Research aimed at elucidating potential candidate genes and characterizing their role on these important traits is an essential preliminary step to incorporate genetic manipulation into future biotechnology projects.

There are two proposed models for the genetic control of complex traits: the infinitesimal model and the major gene model. The infinitesimal model assumes that complex traits are controlled by large numbers of unlinked genes, of which each has only an infinitesimal effect on the trait. In contrast, the major gene model assumes that a small number of major genes contribute a substantial proportion of the genetic variation in the expressed trait. The results from QTL mapping reports suggest that modest numbers of QTL can explain some, but not all of the genetic variation in the complex traits.

In August of 2007, A Johns Hopkins University scientist (Se-Jin Lee) illustrated that the absence of the protein myostatin (MSTN) leads to oversized muscles in mice and reported that a second protein, follistatin, when triggered to overproduce in mice lacking the protein MSTN in turn quadruples the muscle mass (Lee, 2007). Transgenic mice expressing the MSTN pro-domain (Yang et al., 2001; Mitchell and Wall, 2004) also showed significantly increased muscle mass resulting in 22–44% heavier carcasses compared to the controls. They concluded that the lower percentage of fat in those mice was due to a higher proportion of lean mass, because the epididymal fat pad weight was not reduced. The dramatic muscular phenotype, observed throughout the whole carcass, was attributed to muscle hypertrophy since no change in fiber numbers between controls and transgenic mice were detected. Fast-twitch fibers were larger in transgenic mice. Thus, overexpression of the MSTN pro-domain could also be an alternative to MSTN knockout as a means of increasing muscle mass. Researchers at Adelaide University in Australia have identified a gene that they claim explains a large increase in the retail beef yield of edible tissue. While the gene, called MSTN F94L, is not the only gene that influence retail yield, they indicate that it has a tremendous effect on the retail yield.

Bovine

Information in this area is very limited and highly desired by federal agencies that regulate food safety issues. There have been some studies evaluating the meat of animals cloned from embryonic cells (Gerken, Tatum, Morgan, & Smith, 1995; Diles et al., 1996; Harris et al., 1997). Those results, however, do not correspond with the products from animals cloned from adult somatic cells. This is because embryonic animal clones are produced from blastomeres of fertilized embryos at a very early stage of development, and thus embryonic clones may undergo little gene reprogramming during their development. Consequently, they would not serve well as scientific evidence for assessing the food safety risks of somatically cloned food animals. A few reports which provide data on the composition of meat and dairy products derived from adult somatic cell clones indicate that these products are equivalent to those of normal animals. The first report on the chemical composition of bovine meat arising from genetic engineering was in cloned cattle (Takahashi & Ito, 2004). In the meat samples derived from cloned and non-cloned Japanese Black cattle, at the age of 27–28 months, data were collected for proximate analysis (water,

protein, lipids, and ash) as well as fatty acids, amino acids, and cholesterol. The results of this study showed that the nutritional properties of meat from cloned cattle are similar to those of non-cloned animals, and were within the recommended values of the Japanese Dietetic Information guidelines. Also, based on the marbling score, the meat quality score of the cloned cattle in this study graded high (Class 4) according to the Japanese Meat Grading Standard (Class 1, poor to Class 5, premium). No other carcass characteristics were discussed in this report.

A comprehensive study designed specifically to provide the scientific data desired by U.S. regulatory agencies on the safety issue of the composition of meat and milk from animal cloning was recently published (Tian et al., 2005). All animals were subjected to the same diet and management protocols. They analyzed over 100 parameters that compare the composition of meat and milk from beef and dairy cattle derived from cloning, to those of genetic- and breed-matched control animals from conventional reproduction. The beef cattle, in this study, were slaughtered at 26 months of age and also examined for meat quality and carcass composition. A cross section between the sixth and seventh rib of the left side dressed carcass was inspected according to the Japan Meat Grading Association guidelines. Additional parameters of the carcass analyzed were organ or body part weights and the total proportion of muscle and fat tissue to carcass weight. The histopathology of seven organs was examined for appearance of abnormalities. Six muscles (infraspinatus (IS), longissimus thoracis, latissimus dorsi, adductor, biceps femoris (BF), and semitendinosus) were removed from the carcass and measured for the percentages of moisture, crude protein, and crude fat. Samples from these muscles for muscle fiber type profiling, however, were not performed. The fatty acid profile of five major fat tissues (subcutaneous fat, intra- and inter-muscular fats, celom fat, and kidney leaf fat) and the amino acid composition of the longissimus thoracis muscle was also determined. Out of more than 100 parameters examined, a significant difference was observed in 12 parameters for the paired comparisons (clone vs genetic comparator and clone vs breed comparator). Among these 12 parameters, 8 were related to the amount of fat or fatty acids in the meat/fat. The other four parameters that were found different between clones and comparators include yield score, the proportion of longissimus thoracis muscle to body weight, the muscle moisture, and the amount of crude protein in the semitendinosus muscle, all fall within the normal range of industry standards. Therefore, none of these parameters would be a cause for concern to product safety.

The mechanisms of regulation of muscle development, differentiation, and growth are numerous and complex. Meeting the challenge of optimizing the efficiency of muscle growth and meat quality requires a thorough understanding of these processes in the different meat-producing species. Application of biotechnology for livestock and meat production potentially will improve the economics of production, reduce environmental impact of production, improve pathogen resistance, improve meat quality and nutritional content, and allow production of novel products for food, agricultural, and biomedical industries.

In a recent article by Wall et al. (2005), the authors reported the success of genetically enhanced cows with lysostaphin to resist intra-mammary *Staphylococcus*

aureus (mastitis) infection. Mastitis is the most consequential disease in dairy cattle and costs the U.S. dairy industry billions of dollars annually. Their findings indicated that genetic engineering of animals can provide a viable tool for enhancing resistance to the disease and thus improving the well-being of the livestock.

Ovine

Although the first mammalian species to be cloned using a differentiated cell (Wilmut et al., 1997) was ovine, continued development of cloning technology in this species has been in support of conserving endangered species (Loi et al., 2001; Ryder, 2002). About 5–10% of cloned sheep embryos result in offspring, but not all are healthy. Several groups have attempted transgenic introduction of growth hormone (GH) genes in sheep, but none have resulted in commercially useful transgenic animals. Growth promoting TG in sheep was first accomplished by Hammer et al. (1985) followed by Rexroad et al. (1989, 1991) where gene constructs inserted into the sheep produced a 10–20 times elevation of plasma GH level. Growth rates were similar to the control sheep early in life, but after 15–17 weeks of life, the over expression of GH was cited by Ward et al. (1989) and Rexroad et al. (1989) to be responsible for reduced growth rate and shortened life span. Ward et al. (1990) summarized their studies with transgenic sheep, noting reduced carcass fat, elevated metabolic rate and heat production, skeletal abnormalities, and impaired survival due to the unregulated production of GH in the transgenic sheep unless an all ovine construct was used.

The pattern of expression of the various growth hormones and growth-hormone releasing factor (GRF) TG in sheep could not be predicted (Murray and Rexroad, 1991), since circulating levels of GH and insulin-like growth factor I (IGF-I) levels did not correlate to expression of the TG. Transgenic sheep that were non-expressing had transgenic progeny that also failed to express the TG (Murray and Rexroad, 1991). Transgenic lambs which expressed either GH or GRF had growth rates similar to non-transgenic controls, even though the transgenic lambs had elevated plasma levels of IGF-I and insulin. Early literature on transgenic sheep expressing GH indicated similar growth rates and feed efficiency (Rexroad et al., 1989) as non-transgenic controls; however, all transgenic sheep displayed pathologies and shortened life span. Further, transgenic sheep expressing GH, were noted to have significantly reduced amounts of body and perirenal fat (Ward et al., 1990; Nancarrow et al., 1991), and were also susceptible to developing chronically elevated glucose and insulin levels of diabetic conditions.

Progress in overcoming the health problems of GH transgenic sheep was made by switching to an ovine GH gene with an ovine metallothionein promoter (Ward and Brown, 1998). They encountered no health problems through, at least, the first four years of life; although Ward and Brown (1998) noted increased organ sizes and noticeably reduced carcass fat in the G1 generation. Twenty transgenic lambs of the G2 generation (Ward and Brown, 1998) grew significantly faster than the controls,

with differences detected between rams and ewes. Growth rate of transgenic rams was greater than controls from birth onwards; whereas, increased growth rate in transgenic ewes were not noted until 4 months of age. No difference in feed conversion from 4–7 months of age was observed between control and transgenic lambs (Ward and Brown, 1998). In the G3 generation, Brown and Ward (2000) reported the average difference in body weight between transgenic and controls at 12 months of age was 8 and 19% heavier for rams and ewes, respectively. Their results were consistent with the increased circulating levels of GH in the transgenics compared to controls.

Piper, Bell, Ward, and Brown (2001) evaluated the effects of an ovine GH TG on lamb growth and the wool production performance using 62 transgenic Merino sheep. The G4 transgenic lambs were from a single transgenic founder ram and were compared to 46 sibling controls. Pre-weaning body weights were similar for transgenics and controls, but began to diverge and were significantly different from 7 months of age onward. Transgenic lambs were about 15% larger than the controls at 12 months of age and had a very low amount of subcutaneous fat. Major wool production traits, greasy fleece weight and mean fiber diameter, were not different from the controls.

Adams, Briegel, and Ward (2002) also examined the effects of a TG encoding ovine GH and an ovine metallothionein promoter, in the progeny of 69 Merino and 49 Poll Dorset lambs from ewes inseminated by G4 transgenic rams heterozygous for the gene construct. As seen in earlier research using mouse-derived GH transgenes, the effects of the ovine construct varied according to the active expression of the TG. The TG failed to be expressed in some progeny (Adams et al., 2002) despite a positive status for the TG. The ovine GH produced negligible health problems, similar to that reported by Ward and Brown (1998). Among the progeny with active TG expression, plasma GH levels were twice those of the controls. Those sheep also grew faster to heavier weights and were leaner, but had higher parasite fecal egg counts compared to the non-transgenic sheep. Females at 18 months of age had decreased longissimus muscle depth compared to males. Adams et al. (2006) concluded that phenotypic effects of genetic manipulation of sheep may depend on age, breed, and sex of the animal and that modification to the fusion genes is required to meet the species-specific requirements to enhance expression in the transgenic sheep while maintaining the long-term health status.

Callipyge sheep have muscle fiber hypertrophy determined by a paternally inherited polar overdominance allele (Cockett et al., 1994), which is a result of a single base change (Freking et al., 2002; Freking, Smith, & Leymaster, 2004). This naturally occurring mutation that alters the muscle phenotype in sheep was described by Jackson and Green (1993) and Cockett et al. (1994), and since has been subject of much research. The callipyge phenotype is a post-translational effect (Charlier et al., 2001), in which the dam's normal allele suppresses the synthesis of at least four proteins that form muscle tissue. The phenotype is characterized by hypertrophy in certain muscles, vis., longissimus thoracis et lumborum (LTL), gluteus medius, semimembranosus, semitendinosus, adductor, quadriceps femoris, BF, and triceps brachii, while other muscles, such as IS, and supraspinatus (SS),

are unaffected. The hypertrophy is caused by increased size of the fast-twitch fibers rather than increased fiber numbers (Carpenter, Rice, Cockett, & Snowder, 1996). Lorenzen et al. (1997) measured the elevated protein/ DNA ratio in callipyge LTL and BF but not in IS and SS muscles. Fractional protein accretion rate did not differ among those muscles, and protein synthesis rate was decreased by 22% in callipyge LTL and by 16% in callipyge BF muscles. Since the protein degradation rate was also decreased by 35% in callipyge compared to the controls, Lorenzen et al. (1997) concluded that callipyge-induced muscle hypertrophy was due to decreased muscle protein degradation. Reduced tenderness in callipyge was also related to higher calpastatin (CAST) (Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995; Freking et al., 1999; Goodson, Miller, & Savell, 2001) and m-calpain activities (Koohmaraie et al., 1995) compared to the control sheep. Otani et al. (2004) presented an evidence in mice that overexpression of CAST contributes to muscle hypertrophy, although this has not been investigated in relation to the callipyge phenotype.

Busboom et al. (1994) indicated that callipyge lambs had less monounsaturated and more polyunsaturated fatty acids (PUFA) than the controls. Muscle hypertrophy in callipyge sheep was also at the expense of adipose tissue (Rule, Moss, Snowder, & Cockett, 2002), possibly from a decrease in differentiation of the adipocytes. Rule et al. (2002) measured lower lipogenic enzyme activities in adipose tissues of heterozygous callipyge lambs compared to the controls, but were unable to relate these differences to insulin or IGF-I levels. The callipyge locus has been mapped to a chromosome segment that carries four genes that are preferentially expressed in the skeletal muscle and are subject to parental imprinting, namely, Delta-like 1 (DLK1), gene-trap locus 2 (GTL2), paternal expressed gene 11 (PEG11), and maternal expressed gene 8 (MEG8). The same conserved order was found on human and mouse chromosomes. The causative mutation for callipyge is a single base transition from A to G in the inter-gene region between DLK1 and GLT2 (Bidwell et al., 2004). Charlier et al. (2001) demonstrated the unique and very abundant expression of DLK1 (involved in adipogenesis) and PEG11 (unknown function) in callipyge sheep; however, the authors were not able to explain how the over expression of these genes were related to muscle hypertrophy. They suggested that the callipyge mutation does not alter the imprinting of DLK1 or PEG11, but modifies the activity of a common regulatory element which could be an enhancer or silencer. Bidwell et al. (2004) similarly detected elevated DLK1 and PEG11 in the muscles of lambs with the callipyge allele and named them as candidate genes responsible for the skeletal muscle hypertrophy. PEG11 was 200 times higher in heterozygous and 13 times higher in homozygous callipyge sheep than in the controls. Freking et al. (2004) discussed expression profiles and imprint status of genes near the mutated region of the callipyge locus. Markers for polymorphic genes that control fatness and leaness, such as, thyroglobulin, or the callipyge gene, could be used for making genetic selection improvements in animals (Sillence, 2004).

The apparent advantages of higher carcass yield, increased lean and reduced fat content of callipyge sheep would benefit the meat industry except for the associated toughness in the hypertrophied muscles. In contrast to minimal tenderness

improvement using ante-mortem techniques to control growth rate, size, or fatness level (Duckett, Snowder, & Cockett, 2000) or treatment with dietary vitamin D_3 (Wiegand, Parrish, Morrical, & Huff-Lonergan, 2001), some success at improving the tenderness of meat from callipyge has been accomplished by various post-mortem treatments. Tenderness was improved slightly by electrical stimulation (Kerth, Cain, Jackson, Ramsey, & Miller, 1999). Other post-mortem treatments effective for improving the tenderness in callipyge include prerigor freezing prior to aging (Duckett, Klein, Dodson, & Snowder, 1998), calcium chloride injection (Koohmaraie, Shackelford, & Wheeler, 1998), hydrodynamic pressure treatment (Solomon, 1999), and extended aging to 48 days (Kuber et al., 2003). The higher CAST level responsible for the hypertrophy of callipyge lambs (Koohmaraie et al., 1995; Freking et al., 1999; Goodson et al., 2001) is often cited as contributing to the lower tenderness of the meat because CAST interferes with the normal post-mortem proteolysis during aging, particularly the breakdown of troponin-T (Wiegand et al., 2001). The lack of tenderness associated with the callipyge gene must be addressed before the economic advantages can be realized.

Porcine

Among major livestock species, the pig was last to be cloned (Onishi et al., 2000; Polejaeva et al., 2000; Betthauser et al., 2000). There appears to be more interest in transgenesis and cloning of pigs as a model for studying human diseases, such as osteoporosis and diabetes, and for donor organs for xeno-transplantation rather than for improving meat production. Pigs, due to their vast numbers and similar organ size and function like that of humans, are desirable for xeno-transplantation. Hyperacute rejection of xeno-transplanted organs was a major concern until Prather, Hawley, Carter, Lai, and Greenstein (2003) accomplished genetic modification of the (1,3)-galactosyltransferase gene prior to nuclear transfer cloning. Nuclear transfer cloning efficiency rates for swine averages between 1 and 6% of embryos. This and other issues need to be solved with this technology. Cloned pigs appear to have inadequate immune systems (Carroll, Korte, Dowd, & Prather, 2004), display behavioral variations (Archer, Friend, Piedrahita, Nevill, & Walker, 2003), and could transmit viruses (van der Laan et al., 2000). In contrast, Carter et al. (2002) used green fluorescent protein TG and then cloned pigs to evaluate the phenotype and health status. They declared that cloned pigs can be normal and without impaired immune system.

Approximately 40% of the red meat consumed worldwide comes from pigs (FAO, 2004), and pork consumption has increased consistently with increasing world population. Continued improvements in pork production, therefore, are needed to meet future demands for red meat. Research in genomics is one avenue to increase production efficiency. Selection of pigs based on the ranodyne receptor (RyR) gene, muscle regulatory factor (MRF) gene family, hormones, or other potential candidate genes affecting growth and fattening traits are needed to increase

production. QTL evaluation of factors associated with meat quality and growth are underway; however, in pigs, some quality traits are polygenic (Krzecio et al., 2004b) requiring evaluation of their interactions.

In pigs, halothane sensitivity is associated with malignant hyperthermia syndrome and reduced meat quality. Kortz et al. (2004) evaluated meat quality parameters like pH, water binding capacity, water-soluble protein content, and meat color, among other traits to determine the frequency of occurrence of normal vs PSE (pale, soft, exudative) meat quality. Pigs that were recessively homozygous (nn) for halothane sensitivity had higher amount of carcass lean and had higher frequencies of PSE than the dominant homozygous (NN) pigs. The heterozygous genotype (Nn) pigs had the leanest and a lower proportion of carcasses with partial or fully PSE meat. The NN genotype did not guarantee PSE free meat as PSE was also observed in NN carcasses. Milan et al. (2000) related the Rendement Napole (RN) allele, which originated in Hampshire breed of pigs, to 70% increased glycogen content in the muscle and poor water binding quality. Hedegaard et al. (2004) characterized proteome patterns related to the porcine RN– genotype and showed changes in the expression and activity of the key enzymes of glycolysis as well as down-regulation of an intracellular antioxidant enzyme. The RN– mutation likely leads to a loss of function resulting in the reduced degradation of glycogen, based on adenosine monophosphate-activated protein kinase (AMPK) activity which is approximately three times lower in RN– than in normal rn+ pigs (Hedegaard et al., 2004). The RN– allele is of interest to pig breeders because it is also associated with increased growth rate and lean content in the carcass. The negative outcome of this mutation, however, is lower 24 hours post-mortem muscle pH, reduced water binding capacity, and reduced cooked ham yields. The RN– was mapped to a mutation, coined PRKAG3, which is the third isoform identified of a mammalian AMPK. AMPK plays a central role in regulating energy metabolism through glucose transport into the cell and in fatty acid synthesis and oxidation. The muscle-specific expression of PRKAG3 is consistent with the fact that RN– pigs have high glycogen content in their muscles but not in the liver. The PRKAG3 mutation was identified by seven nucleotide differences between rn+/rn+ and RN–/RN– pigs. Analysis of the single nucleotide polymorphisms further identified the 200 codon region to be the causative polymorphism. This 200Q substitution was found in RN– pigs but not in any rn+ pigs. Functional characterization of the RN– mutation is complicated by its location in a regulatory subunit of AMPK and by the expression of several isoforms of AMPK in skeletal muscle. Completion of the porcine genome sequence will increase the identification of genes and interactions with other genes associated with controlling muscle and fat. Transgenesis to inhibit or increase the action of these genes may prove useful in increasing pork production.

QTL analysis of factors affecting tenderness and juiciness of the pork were mapped to chromosome 2, and based on that location the CAST gene was considered (Ciobanu et al., 2004) a likely candidate. Meat quality traits in pigs negative for the halothane sensitivity ryanodyne receptor (RyR1) and RN– alleles were evaluated for interactions with CAST (Krzecio, Kury, Kocwin-Podsiada, & Monin, 2004a). For stress-resistant RyR1 pigs, CAST polymorphisms using the Rsa1 restriction

enzyme (CAST/Rsa1) were identified as AA, AB, and BB genotypes. These were found to affect water holding capacity (WHC), drip loss, and water and protein content of the muscle. CAST/Rsa1 AA genotype pigs had lower WHC, lower drip loss at 96 hours, less moisture and higher protein content in muscle compared to BB genotype. Stress resistant pigs (homozygous and heterozygous RyR1 resistant genotype) had highly significant lactate level, pH at 35 and 45 minutes post-mortem and on reflectance values. Homozygous stress resistant pigs produced the most desirable quality traits. The interaction of CAST/Rsa1 and RyR1 was significant for the longissimus lumborum muscle pH at 45 minutes post-mortem and drip loss at 48 h; however, no interactions were detected for carcass lean (Krzecio et al., 2004a, 2004b) or cooking yield. That CAST and RyR1 would interact is not surprising since CAST is an endogenous inhibitor of calcium-dependent cysteine proteases, the calpains, and a mutation in RyR1 is partly responsible for the disturbed regulation of intracellular Ca^{2+} in pig skeletal muscle (Kuryl, Krzecio, Kocwin-Podsiada, & Monin, 2004). These studies indicate that the quality of meat should be considered not only by each individual genotype, but also by the interactions with other genes.

Polymorphisms of the CAST gene and their association between genotypes at the porcine loci MSTN growth differentiation factor 8 were considered by Klosowska et al. (2005). Mutations in the MSTN gene are responsible for extreme muscle hypertrophy, or double muscling, in several breeds of cattle. MSTN is important for controlling the development of muscle fibers and is considered to be a negative regulator of muscle growth (McPherron, Lawler, & Lee, 1997). Since calpain activity is required for myoblast fusion, cell proliferation and growth, it may also affect the number of skeletal muscle fibers. The fusion of myoblasts to form fibers is accompanied by a dramatic change in the calpain/CAST ratio. Over expression of CAST, an endogenous calpain inhibitor in transgenic mice resulted in substantially increased muscle tissue (Otani et al., 2004). Klosowska et al. (2005) analyzed the interaction of MSTN and CAST in Piétrain × (Polish Large White × Polish Landrace) cross-bred pigs and the Stamboek line of Dutch Large White × Dutch Landrace pigs. The MSTN genotypes identified using the Taq1 restriction enzyme were CC or CT, and CAST/Rsa1 genotypes were identified as EE, EF, or FF. They reported that 79.5% of the Stamboek line was characterized as MSTN/Taq1 CC genotype. Interestingly, the FF genotype of CAST/Rsa1 was not detected in the Piétrain cross-bred pigs. Muscle fiber size and type distributions were not affected by the MSTN genotypes although there were breed differences. Piétrain crosses had larger mean fiber diameters in all the fiber types compared to Stamboek pigs. Proportion of fiber types in a bundle was higher for slow-twitch oxidative (SO) and lower for fast-twitch glycolytic (FG) fibers in Piétrain cross-bred pigs compared to Stamboek pigs. Of the multiple deletions or substitutions identified for MSTN, only one results in muscle hypertrophy seen in double muscle cattle and in mice. The C to T replacement in the MSTN gene does not result in an amino acid substitution (Stratil and Kopecny, 1999), thus it is probable that this genotype has no effect on the MSTN function in pigs. Muscle fiber diameters and the number of fibers per unit area were not different for CAST genotypes in Piétrain cross pigs, whereas,

the CAST genotype had an effect in the Stamboek line. In all the fiber types, fiber diameters were larger in the CAST EE and EF genotypes and smallest in FF. Loin eye area of EE genotype also was significantly larger than for EF or FF genotypes. Because of the missing FF genotype in Piétrain cross pigs, the interaction of CAST and MSTN could not be assessed.

Transgenic pigs expressing a plant gene, spinach desaturase, for the synthesis of the essential PUFAs, linoleic and linolenic acids, have been produced (Saeki et al., 2004), marking the first time that a plant gene has been functionally expressed in mammalian tissue. This transgenesis could result in a significant improvement in pork quality beneficial to human health. They detected levels of linoleic acid in adipocytes that was about ten times higher in transgenic than in the control pigs. Niemann (2004) suggested that modifying the fatty acid composition of products from domestic animals may make this technology more appealing to the public. High levels of dietary PUFA were shown to improve processing and increased PUFA in pork muscle. Earlier work with transgenic pigs and with injected porcine somatotropin also led to reduced levels of saturated fatty acids in pork (Pursel and Solomon, 1993; Solomon, Pursel, & Mitchell, 2002).

Many reports have documented the effects on growth of pigs receiving additional GH by exogenous administration or endogenously through transgenesis (Vize et al., 1988; Wieghart et al.,1988; Pursel et al., 1988; Pursel and Rexroad, 1993; Pursel and Solomon, 1993; Pursel et al., 1997; Solomon, Pursel, Paroczay, & Bolt, 1994). Transgenic pigs expressing IGF-I, a regulator of GH, have been described in detail (Solomon et al., 2002; Mitchell and Pursel, 2003; Pursel et al., 2001a, 2001b, 2004). Pursel et al. (2004) summarized the advances made in pigs expressing a skeletal α-actinin-hIGF-I TG, namely, the expression of IGF-I in skeletal muscles gradually improved body composition in transgenic pigs without major effects on growth performance. Lean tissue accretion rates were significantly higher (30.3 and 31.6%), and fat accretion rates were 20.7 and 23.7% lower in transgenic gilts and boars, respectively, compared to controls. Body fat, bone, and lean tissue measurements by dual-energy X-ray absorptiometry confirmed that transgenic pigs had less fat and bone but higher lean tissue amount than the control pigs.

Dietary conjugated linolenic acid (CLA) and IGF-I TG had little or no effect on pork quality (Eastridge, Solomon,Pursel, Mitchell, & Arguello, 2001; Solomon et al., 2002). Carcass weight of IGF-I TG pigs was less than non-TG controls; however, TG pigs had a 16% larger loin eye area, 26–28% reduced back fat thickness, and 21% less carcass fat. Dietary CLA acted synergistically with the IGF-I TG in reducing back fat thickness. Muscle pH at 45 minutes (pH_{45}) was lower ($p < 0.01$) in TG than non-TG (6.0 vs 6.1), while dietary CLA resulted in significantly higher pH_{45} than for pigs fed with control diets (pH_{45} 6.1 vs 6.0). At 24 hours, muscle pH was not different, averaging pH 5.6, for all carcasses. Neither the gene status nor dietary CLA affected drip/purge loss during the 21 days refrigerated storage in a vacuum package, pork chop cooking yield, or thiobarbituric reactive substances measured in vacuum packaged loins stored for 5 and 21 days fresh and 6 months frozen. In pigs receiving the control diet, pork chop tenderness was improved significantly,i.e., lower shear force values, in IGF-I TG compared

to non-TG (5.3 vs 7.0 kgf). Dietary CLA improved the tenderness in non-TG pigs equivalent to the tenderness of TG. Wiegand et al. (2001) detected no effects of CLA supplementation of swine diets on sensory attributes; although, it improved meat color, marbling, and firmness. Bee (2001) detected no effect of CLA on pig growth performance, carcass lean, or fat deposition, but there was a marked effect on fatty acid profiles. Saturated fatty acids, palmitic and stearic, were increased significantly while monounsaturated linoleic and polyunsaturated arachidonic acids were reduced. Activity of lipogenic enzymes in vitro was not altered by the dietary CLA suggesting that lipogenesis was not affected by CLA (Bee, 2001).

Directing IGF-I expression specifically to skeletal muscle appeared to overcome the problems encountered with GH transgenics or with daily injections of exogenous IGF-I (Pursel et al., 2004) and clearly had a major impact on carcass composition. Piétrain pigs have 5–10% more meat than comparable pigs of other breeds (Houba and te Pas, 2004), although the muscle hypertrophy phenotype in Piétrain pigs is not as strongly expressed as the double-muscle condition in cattle or callipyge in sheep. The mechanism of Piétrain pig hypertrophy is still unknown; however, it may be associated with changes to the CAST gene. Klosowska et al. (2005) did not detect a CAST polymorphism FF genotype in Piétrain cross-bred pigs. Pigs with the FF CAST genotype had smaller muscle fiber diameters compared to the EE and EF phenotypes. Linking the CAST genotype with phenotype to meat quality would benefit the meat industry, especially in pigs. The relationship between the genotype at the CAST and MSTN loci to phenotype remains to be elucidated.

Conclusions

The development of recombinant DNA technology has enabled scientists to isolate single genes, analyze and modify their nucleotide structure(s), make copies of these isolated genes, and insert copies of these genes into the genome of plants and animals. The transgenic technology of adding genes to livestock species has been widely adopted because it is technically straightforward, although it is not efficient. The primary goal of transgenesis is to establish a new genetic line of animals, in which the trait(s) of concern are stably transmitted to succeeding generations. Not all injected eggs will develop into transgenic animals and not all transgenic animals will express the TG in the desired manner. Eating quality and food safety must not be compromised as meat animals are designed and developed using these biotechnological approaches.

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