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REDEFINING BODY COMPOSITION: Nutrients, Hormones, and Genes in Meat Production[1]

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ABSTRACT

Growth rate and body composition of livestock can be optimized to meet consumer needs for a leaner product and to improve the efficiency of meat-animal production. Optimization strategies have traditionally focused on genetic selection and cost-effective ration formulation to achieve the genetic potential. Advances in understanding the mechanisms of growth and its control have led to additional opportunities for its manipulation. These include nutritional manipulation, the use of growth promotants, and, more recently, the ability to change the genetic potential through genetic engineering. Selection of appropriate candidate genes for manipulation depends on understanding the mechanisms underlying differentiation and growth of embryonic muscle cells. Recent advances in genetic engineering techniques, including gene therapy and germline transgenesis, will likely hasten the genetic progress toward a leaner carcass in domestic livestock. Such strategies may prove to be more beneficial than the controlled enhancement of somatotropin expression.

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CONTENTS

INTRODUCTION

Production of livestock for milk and meat represents 50% of total agricultural income and is the most value-added outlet for the bountiful grain and oilseed produced in the United States (134). Depending on the species, 50–70% of production costs are associated with feed. Therefore, much research on livestock has been aimed at maximizing feed-conversion efficiency. The feed-conversion ratio represents the interplay between the rate of body gain, the nature of tissue deposited, and the inefficiencies of digestive processes. This complexity makes possible several quite different strategies to improve conversion efficiency.

In this review, current understanding of livestock growth and development is summarized, including possible techniques for manipulating these processes. We emphasize the central role regulation of the somatotropin/insulin-like growth factor axis plays in nutrient partitioning and feed-conversion efficiency. The interplay between somatotrophic endocrine (anabolic) signals and catabolic signals, including immune system cytokines, is shown in Figure 1 (52). For a recent review of the biology of somatotropin in livestock, see Etherton & Bauman (54).

Regardless of strategy, all procedures influencing growth and development affect the priority of nutrient partitioning by body tissue pools, as originally proposed by Hammond (72). Muscle and adipose tissue rank relatively low in priority in the body's allocation of nutrients compared with bone, lymphoid, and neural tissues. Currently, most strategies for growth manipulation attempt to increase the nutrient allocation priority for muscle tissue deposition; these strategies concomitantly reduce adipose tissue growth. Biotechnology offers promising new strategies for achieving the desired partitioning of nutrients. Recent advances in the understanding of signal transduction pathways at the target tissue level may focus research interests in this direction, away from the manipulation of primary endocrine signals.

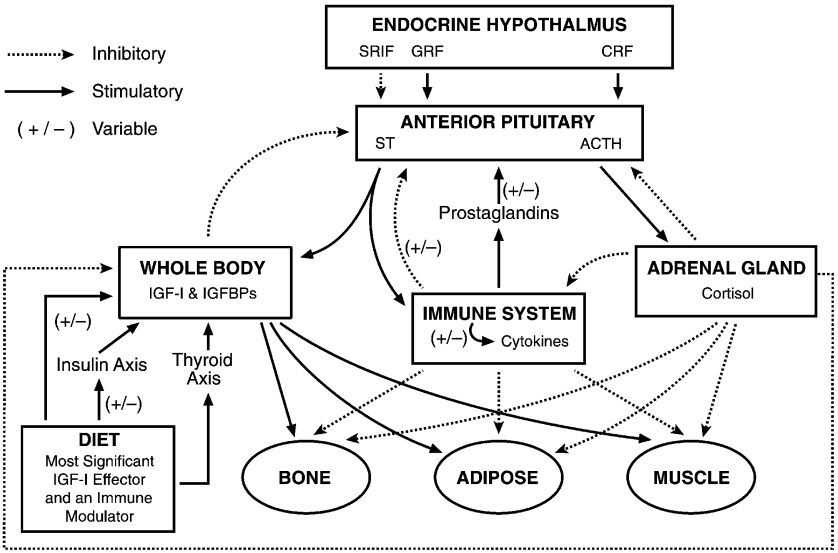

Figure 1 This diagram illustrates some of the interactions between the endocrine signals that stimulate growth and maintain tissue stability (somatotropic axis: ST and IGF-I) and the endocrine (glucocorticoids) and cytokine (TNF) systems that mobilize stored tissue products and divert nutrients from growth. The conflict or balance between these systems determines to what extent nutrients are diverted away from growth processes to address an imposed stress. SRIF, Somatostatin; GRF, growth hormone releasing factor; CRF, corticotropin-releasing factor; ST, somatotropin; ACTH, adrenocorticotropic hormone; IGF-I, insulin-like growth factor-I; IGFBPs, IGF-binding proteins; TNF, tumor necrosis factor. (Adapted from Reference 52.)

CANDIDATES FOR CONTROL IN THE PRENATAL ENVIRONMENT

Stages of Muscle Development

All skeletal muscle is derived from somite cells. These cells rapidly differentiate and migrate in order to found premuscle masses. These migrating cells, or myoblasts, are predetermined and can form only muscle (18). After migration, these embryonic myoblasts initially proliferate and then fuse to form differentiated, multinucleated myotubes that then begin expressing genes for contractile proteins. Fusion of proliferating mononucleated myoblasts is a terminal step in muscle differentiation—once nuclei have been incorporated into a myotube, they are no longer capable of division. Near birth, the myotubes mature into functional myofibers (Figure 2). During postnatal growth, new myonuclei will be added to the myofiber from satellite cells, which fuse with the muscle fiber to increase its length.

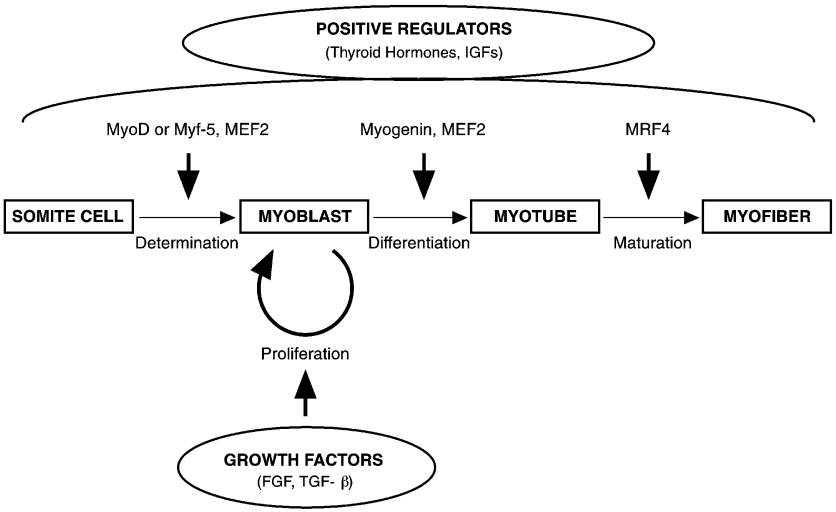

Figure 2 Outline of the main events and regulatory factors involved in myogenesis and differentiation. This diagram highlights some of the points at which specific regulators act. Muscle-specific transcription factors include (*a*) the myogenic determination factor (MyoD) family, which is comprised of MyoD, Myf-5, myogenin, and muscle regulatory factor (MRF4), and (*b*) myocyte-specific enhancer-binding factor 2 (MEF2). Positive regulators act at many stages of development, for example by inducing myoblasts to exit from the cell cycle; many growth factors, such as fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) act antagonistically by preventing cell cycle arrest and repressing myogenic gene expression. IGFs, Insulin-like growth factors. [Adapted and reproduced with permission from Dauncey & Gilmour (38).]

Myogenesis in pigs, as in many mammalian species, is a multiphasic phenomenon with the sequential formation of at least two generations of muscle fibers. A primary generation forms between 35 and 55 days of gestation, followed by a second generation that forms between 60 and 90 days (94). The total number of muscle fibers is generally considered to be established by 90–95 days of the 114-day gestation. Recently, a third generation of small-diameter fibers has been observed shortly after birth in both pigs (99) and sheep (169). From late gestation through the first few postnatal weeks, the myotubes mature into highly organized myofibers (95).

Regulation of Myogenic Proliferation and Differentiation

Regulation of the proliferation and differentiation of both satellite cells and embryonic myogenic cells is crucial to the development of muscle tissue in domestic animals. Current evidence suggests that the myogenesis determination

factor (MyoD) family of proteins and three growth factor families—the insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), and transforming growth factor-β (TGF-β)—play major roles in this regulatory process (Figure 2).

The discovery of the MyoD family of genes has revolutionized the study of growth and differentiation of muscle cells. Members of the MyoD family of proteins [MyoD, myogenin, myf-5, and muscle regulatory factor-4 (MRF4)] activate the transcription of many skeletal muscle genes and are so named because of their remarkable abilities to impose a myogenic fate on nonmuscle cells (167). The function of the MyoD family of myogenic regulators has been addressed by gene-knockout experiments in mice. Mice bearing targeted null gene mutations of either the myf-5 or MyoD gene do form skeletal muscle; however, when both factors are absent, mice lack muscle as well as the precursor myoblast population (145). These results indicate that both of these two factors may not be required for myogenesis, but they clearly point to a requirement for one or the other in myoblast determination. Myogenin-knockout mice exhibit myoblasts, but very few muscle fibers form (76). Thus, myogenin is important for myogenic differentiation and acts downstream of myf-5 or MyoD. MRF4 is usually expressed in older, differentiated muscle fibers (110). MRF-4 knockout mice show varying phenotypes, depending on the degree of alteration in the nearby myf-5 gene. Relatively subtle phenotypic changes such as up-regulation of myogenin and modest reductions in deep back and intercostal muscles late in gestation result from knocking out MRF4 alone (177). Another member of the MyoD family, myocyte-specific enhancer factor-2 (MEF-2), is an intermediate myogenic regulatory factor that is induced by myogenin and MyoD in skeletal muscle (35). The regulation of MEF-2 and the molecular mechanisms by which it activates skeletal muscle genes are currently being elucidated.

Little is known about the roles of members of the MyoD family in postnatal muscle development. MRF4 is the most abundant transcript in postnatal muscle, indicating that it probably plays a role in the maintenance of transcription of muscle genes. Postnatally, myogenin and MyoD transcripts are detectable, but only at low levels (142), and their mRNAs are preferentially expressed in adult rats in slow, oxidative myofibers and fast, glycolytic myofibers, respectively (79). These results suggest that the MyoD family may mediate fiber type–specific gene expression in mature muscle.

In addition to the MyoD family of proteins, proliferation and differentiation of myogenic cells in embryonic and postnatal muscle are also controlled through the integrated action of stimulatory and inhibitory growth factors. IGFs stimulate both proliferation and differentiation. FGFs stimulate proliferation and inhibit differentiation of most cultured myogenic cells. TGF-β also inhibits differentiation but has conflicting effects on proliferation.

IGFs are unique among mitogens in that they stimulate both muscle cell proliferation and differentiation in many cell types, including muscle cells [reviewed by Florini (60)]. It was recently discovered by Coolican et al (33) that there are two separate signaling pathways from the type I receptor: the MAP kinase pathway, for proliferation; and the phosphatidylinositol 3-kinase pathway, for differentiation. This helps explain how seemingly opposing functions are possible.

The biological functions of IGFs and their receptors in myogenesis have been characterized by gene knockout experiments and transgenic mice studies [reviewed by Wood (171)]. At birth, body weights of both IGF-I– and IGF-II–deficient mice (42) are 40% less than those of wild-type littermates; this prenatal growth retardation is maintained into adulthood, and their muscles are highly underdeveloped. Transgenic mice that overexpress IGF-I have increased masses of muscle and connective tissue (100). When overexpression of IGF-I is specifically targeted to muscle using the promoter for avian skeletal α-actin, transgenic mice exhibit a 47-fold increase in IGF concentrations in skeletal muscle only; this leads to muscle hypertrophy of all myofiber types (32). This demonstrates the importance of autocrine/paracrine actions of IGF-I and is in agreement with studies that used immunoneutralization techniques to determine the role of circulating IGF-I in animal growth, where growth rate was unaffected by passive immunization against IGF-I (86, 156). Together, these results demonstrate that IGFs have significant roles in growth of both fetal muscle and the overall body through autocrine/paracrine mechanisms.

The importance of IGFs to muscle development is further emphasized by the fact that IGF-I–knockout mice are similar to myogenin-knockout mice in that there is essentially no muscle development. This can be explained by the fact that IGFs stimulate differentiation in muscle cells, at least in part, by inducing the transcription of myogenin (62). After myogenin induction, IGF-II expression becomes elevated. Endogenously produced IGF-II then stimulates myogenic differentiation (16). IGF-II also is a critical survival factor during the transition from proliferating to differentiating myoblasts (158).

Mice lacking a functional type I IGF receptor are half normal size and exhibit general organ and muscle hypoplasia (96). In contrast, overexpression of the type I receptor in a primary fetal bovine myogenic culture resulted in elevated rates of IGF-I–stimulated proliferation and terminal differentiation (135), suggesting that manipulation of receptor density in myogenic cells could be a strategy to increase muscle mass in meat-animal production. Mice lacking a functional type II IGF receptor are 30% larger than normal littermates (91) and have increased concentrations of IGF-II, but they die soon after birth. These results are consistent with the hypothesis that at least one function of the type II receptor is internalization and degradation of IGF-II, preventing the accumulation of lethal levels in the circulation.

The biological activity of the IGFs in muscle is modulated by at least three IGF binding proteins (IGFBPs) that are secreted by myoblasts [reviewed by Florini et al (61)]. Both IGFBP-4 and -6 inhibit myoblast proliferation and differentiation. IGFBP-5 inhibits myogenesis in the presence of IGF-II but stimulates differentiation in the presence of IGF-I. Several other factors also regulate IGFBP secretion in muscle cells. TGF-β causes an overall decrease in IGFBPs from L6 myoblasts, whereas FGF stimulates the secretion of IGFBP-4 and -5 from the same cell type (103).

Removal of FGF from culture medium of proliferating myogenic cells arrests proliferation in the G_1 phase of the cell cycle (31), and irreversibly commits the cells to terminal differentiation. Readdition of FGF to the medium does not inhibit this differentiation, possibly because, at least in cell lines, FGF receptors are permanently lost during terminal differentiation (119). In vivo studies, although few, have indicated that FGF levels in the chick embryo limb bud are high enough to inhibit myoblast differentiation (151). The mechanism by which FGF inhibits differentiation is unclear; however, Brunetti & Goldfine (17) reported that FGF suppresses expression of MyoD and myogenin. In treatment of muscle cell cultures, FGF also inhibits IGF-II gene expression and subsequent autocrine secretion of IGF-II (144). Postnatally, FGF is stored in the extracellular matrix of muscle (176) and could play a role in regulating muscle hypertrophy.

TGF-β is a potent inhibitor of differentiation of cultured muscle cell lines, embryonic muscle cells, and adult satellite cells (41). Like FGF, it acts on an early, irreversible step that leads to terminal differentiation (118), its receptors disappear from numerous muscle cell lines as they differentiate (56), and it suppresses the expression of MyoD (160) and myogenin (15). Although there is agreement that TGF-β inhibits myogenic cell differentiation, there is no consensus as to its effects on proliferation. TGF-β has a slight or no stimulatory effect on proliferation of myogenic cell lines and embryonic muscle cells (41). In contrast, other researchers report that TGF-β inhibits proliferation of embryonic porcine myogenic cells (124) and myoblasts isolated from neonatal rats (1). Decreases in local concentrations of both TGF-β and FGF may prevent differentiation of myoblasts during embryonic development. These factors also may regulate fusion of satellite cells in embryonic and postnatal muscle tissue.

The recently discovered member of the TGF-β family, myostatin [also referred to as growth/differentiation factor-8 (GDF-8)], functions specifically as an inhibitor of skeletal muscle growth. Myostatin is first expressed in developing somites (107). Later expression is restricted to developing skeletal muscle, and it continues to be expressed in adult muscle. Two different breeds of heavily muscled cattle, Belgian Blue and Piedmontese (82), have mutations in the myostatin gene. Thus, inhibiting myostatin function may have useful applications in meat-animal production.

The thyroid hormones also stimulate myogenic differentiation. Treatment of the C2 cell line with T_3 induced the expression of MyoD and myotube formation (28). Additionally, thyroid hormone response elements have been characterized in the myogenin gene (47).

Maternal Influences

The maternal environment has a profound impact on the development of the fetus and can impact postnatal growth and the composition of body weight gain. There are two main ways in which the maternal environment can affect the growth of the fetus: by controlling the supply of nutrients to the placenta and the fetus, and by altering the hormonal environment. The fetus is relatively well protected from small fluctuations in its hormonal and nutrient environment, but adequate nutrient supply and hormonal balance (fetal and maternal) are important to its development and affect its body composition at birth and in postnatal life.

NUTRIENT ENVIRONMENT OF THE FETUS Nutrient supply to the fetus is controlled by the dam and the placenta. Restricting feed and/or protein intake to the dam during gestation can alter the rate and the composition of postnatal growth. In most instances, birth weight and postnatal growth are reduced when either feed- (48, 116, 128c,d) or severe protein-restriction (128b, 129a, 150a) is imposed on the dam. Protein deposition has a higher priority than adipose deposition has, and when feed intake to the dam is restricted, progeny have a higher lean-to-fat ratio, primarily because of a reduction in fetal adipose tissue (128c,d). In contrast, when protein is limiting, the fetus must reduce its protein accretion rate. Progeny of protein-restricted dams have a lower lean-to-fat ratio because of a reduction in lean mass with little effect on adipose (128b, 129a, 150a). The reduced protein mass may be due to an increase in protein degradation, as protein restriction during gestation does not alter fractional rates of tissue protein synthesis in skeletal muscle, heart, liver, pancreas, or jejunum of newborn pigs (40). Maternal nutrition can also affect muscle fiber development. Increasing feed intake in pregnant sows can increase both fetal muscle fiber number and the ratio of secondary to primary fibers in the progeny, whereas reduction in lean tissue from maternal nutrient restriction is associated with a decrease in secondary muscle fibers (48).

Severe maternal feed restriction reduces the weights of vital organs such as the liver in the fetus, but it does not affect the weight of high-priority organs such as the brain (131). It can also cause permanent stunting of body weight in the progeny (130). These alterations in mature size may reflect the inability of an animal nutritionally deprived in utero to respond to nutrient intake in a way that efficiently accrues lean tissue mass; an animal with a smaller mature size

will tend to be fatter at a given body weight. Although the ruminant is more resistant to the effects of gestational feed restriction, severe feed restriction can also affect its progeny (10, 116).

Maternal intake also affects the endocrine status of both the dam and the fetus. Gestational protein restriction decreases plasma insulin levels (2) and increases adipose insulin receptors in progeny (122). Protein- and energy-restricted pregnant ewes and their fetuses have elevated somatotropin (ST) levels, and their responses to growth hormone releasing factor (GRF) are enhanced (8). However, the elevated ST levels do not translate into increases in IGF-I. On the contrary, maternal feed restriction reduces both fetal and maternal levels of IGF-I (8). These changes in fetal hormonal status caused by maternal feed restriction may adapt the fetus to an anticipated period of postnatal nutritional deprivation but favor fat rather than lean accretion with adequate postnatal nutrition.

Less is known about the effects of individual nutrients on fetal growth and composition of body weight gain. However, like offspring of diabetic mothers, fetuses from dams with high maternal glucose (84) have a higher lipid content; this is correlated with fetal levels of insulin. However, increasing maternal energy intake over the last 2 weeks of gestation in swine has no effect on the progeny (129). Determining the effects of high maternal glucose is difficult because results are often confounded by alterations in the hormonal status of both the dam and the fetus.

HORMONAL ENVIRONMENT OF THE FETUS The effects of hormonal environment on the fetus has been approached in two ways: administration of exogenous hormones to the dam and/or fetus, and removal of endogenous fetal hormones. In swine, fetal decapitation has been utilized as a means of examining fetal growth in the absence of fetal pituitary hormones. However, this technique also eliminates neural signals from the brain. Decapitated pig fetuses have body and muscle weights similar to those of control pigs, but the muscle tissue of decapitated fetuses is less mature than that of control animals and decapitated fetuses tend to be fatter. Early skeletal muscle development and growth in the fetal pig proceeds relatively normally in the absence of the brain, hypothalamus, and pituitary, but hypothalamic and pituitary hormones and/or neural stimuli are required for muscle maturation (98). Fetal decapitation affects adipose development with increases in total body lipid content and percentage of lipid (83) and altered adipose structure (77). In decapitated fetuses, hepatic fatty acid esterification and synthesis are more active, and de novo fatty acid synthesis in adipose tissue is higher than in intact fetuses (83).

Non-pituitary hormones are also affected by fetal decapitation. Increased pancreatic secretion of insulin in decapitated fetuses may be an important cause of enhanced lipid deposition in the decapitated fetus. Kasser et al (83) suggest

that neural or hypothalamic and pituitary hormonal events may limit adipose deposition in the fetus; decapitation may remove this inhibition.

Circulating levels of insulin in fetuses with intact pituitaries are positively correlated with body weight. This hyperinsulinemia can increase placental weight (63) and may contribute to the increase in fetal body weight, as the placenta can limit fetal growth (10). However, chronic hyperinsulinemia does not always affect fetal body composition in pigs (66), even though hypoinsulinemia is associated with decreased fetal growth (63). This suggests a permissive, rather than stimulatory, role for insulin in fetal growth. Maternal insulin may also have a role in growth regulation and the regulation of IGF-I because treatment of the dam with insulin tends to increase fetal as well as maternal IGF-I (117).

Treatment of the dam with ST in early gestation induces the formation of more fetal skeletal muscle fibers, resulting in a higher growth capacity for skeletal muscle (140). When ST is given during late gestation, the progeny weigh more at birth and also have more muscle fibers per cluster, possibly indicating a higher level of maturity at birth. Increasing maternal ST via GRF administration or immunization against somatostatin (SRIF) results in offspring with a higher protein content, but a combination of the two reduces protein content of piglets (57). However, these effects do not appear to be mediated by increases in fetal IGF-I because administration of ST to pregnant sows does not increase fetal concentrations of IGF-I (117).

Gender also has an impact on body composition. In general, male animals are leaner than females. This is largely due to differences in levels of testosterone. If one could program females in utero with exposure to male sex hormones, leaner females might result. This has been attempted in sheep and cattle, but the results have been mixed. Androgenized heifers (43, 141) and ewe lambs (74, 80) grow faster than nonandrogenized control females, but the effects on body composition and feed efficiency are variable. In utero exposure to male sex hormones can improve feed efficiency (43, 80, 141) and reduce fatness (43, 80), but efficiency and body composition are not always altered (74). In utero exposure to β-adrenergic agonists has not been as promising, and in utero exposure of lambs to the β-adrenergic agonist L644,969 has no effect on growth parameters (152).

CANDIDATES FOR CONTROL IN THE POSTNATAL ENVIRONMENT

Nutrients and Growth Factors in Milk

The dam's influence on her progeny extends into the neonatal period via her milk. As long as intake is not limiting, sow-reared pigs grow more rapidly than

those fed sow's-milk replacer from birth, and colostrum appears to be necessary for the development of tertiary muscle fibers in pigs (38). Calves fed colostrum at birth have higher IGF-I levels than calves fed only milk replacer (71) or those from which colostrum is withheld for 24 hr (69). Milk replacer does not contain growth factors, and colostrum is also much higher in IGF-I than is mature milk. The effects of milk-borne IGF-I on neonatal development have been recently reviewed (20, 46). IGF-I and other milk-borne growth factors may be especially important in growth-compromised animals (e.g. runts and those whose growth was restricted in utero). Runt piglets are born with lower endogenous IGF-I levels, which increase with feeding (143a). Piglets from protein-restricted dams weigh 10% less and have 30% lower IGF-I levels at birth and during the first 2 weeks of life. IGF-I and other milk-borne growth factors may be especially important in growth-compromised animals (e.g. runts), which have lower endogenous IGF-I levels. At birth, piglets from protein-restricted dams weigh 10% less and have 30% lower IGF-I levels.

Infusing IGF-I into the neonatal offspring of restricted dams increases circulating IGF-I concentrations, growth rate, and protein and fat accretion rates to control levels without altering insulin, glucose, IGF-II, or thyroid hormone concentrations (150).

Altering Feed Intake

Different breeds have varying nutrient requirements for maximizing lean tissue gain. Provided the amino acid requirements are met, diet composition has little influence on body composition (108). According to Hammond (72), lean tissue accretion has a higher priority than adipose tissue in the young, growing animal. Therefore, postnatal restriction of intake should alter body composition because it limits lipid accretion before affecting protein accretion. Animals allowed ad libitum access to a balanced diet will overconsume feed and become obese (53, 113), while restricting feed intake results in a leaner body composition (12, 22, 58, 108, 112, 161). When an animal's feed intake is restricted, usually both protein and lipid deposition rates are reduced. However, because the lipid deposition rate is suppressed more than the protein deposition rate, energy restriction results in a leaner animal (138). This effect is most pronounced in the neonate, emphasizing its predisposition toward protein accretion. Even at intakes resulting in negative energy balance, young pigs are able to maintain protein deposition, albeit at a reduced rate (138). The differences in body composition caused by early feed restriction are maintained even after pigs are returned to ad libitum access (11, 12). Except in obese genetic lines (128a), restricting protein intake reduces indices of lean mass and lowers the lean-to-fat ratio (23, 49, 67, 89a, 115a, 128a). Restricting protein intake in early life may limit the pig's ability to retain protein later (23). The propensity for lean

growth does not appear to be as strong in young ruminants as in pigs (67). In suckling goat kids, plane of nutrition and level of intake determine how rapidly the animal grows, but body composition at a given weight is unaffected (148). As might be predicted by the Hammond model (72), one outcome of selection for leaner animals at a given slaughter weight (especially in ruminants) has been a tendency toward a larger mature size (22, 121). An animal with a larger mature size achieves slaughter weight at a younger stage of maturity and is therefore leaner.

Body composition can also be altered by affecting the protein accretion rate, the difference between the rates of protein synthesis and degradation. Increases in the protein accretion rate can occur by differentially affecting protein synthesis and degradation. Reeds and co-workers reviewed the effects of various hormonal and nutritional factors on the rates of protein synthesis and degradation (136–138). Protein restriction reduces protein accretion rates by decreasing the rate of protein synthesis more than it decreases the rate of protein degradation. This results in a decrease in the lean-to-fat ratio, but the effects (at least in neonatal pigs) are not uniform across tissues. In protein-restricted pigs, the proportion of amino acids partitioned to gastrointestinal tissue is preserved, whereas the proportion partitioned to skeletal muscle is reduced (49). During chronic protein restriction, both amino acid absorption (49) and insulin concentrations (3) are reduced, as is the insulin response to feeding (49). This may reduce substrate availability and the anabolic stimulus for muscle protein accretion (49). The act of eating can stimulate muscle protein synthesis in neonates (136), and this appears, in part, to be mediated by insulin (172, 174). However, obese pigs release more insulin in response to feeding but do not have elevated protein accretion rates (105), whereas feed-restricted pigs have a reduced insulin response to glucose, possibly indicative of a reduced lipogenic rate (161). Therefore, nutrient balance and genetics may play roles in altering the sensitivity of individual tissues to anabolic hormones.

Hormone Patterns and Effects of Development

While fetal growth is relatively pituitary independent, pituitary hormones play an important role in postnatal growth and in determining body composition. This is primarily driven by the ST-IGF axis. Even though current dogma maintains that ST is not important in regulating fetal growth, an intact pituitary (and presumably ST) is essential for normal development of pig muscle tissue in the last trimester (25), and ST concentrations are considerably higher in fetal sheep (6) and pigs (7) than in postnatal animals. In fetal sheep, this is partially due to pituitary insensitivity to SRIF (45); this lasts until a few days prior to birth. However, ST receptor mRNA is expressed at high concentrations in fetal pig (149) and fetal sheep (89) skeletal muscle early in the third trimester, coincident

with initiation of myocyte differentiation. Additionally, in the fetal pig, skeletal muscle specifically binds ST, which suggests that receptor mRNA is translated into receptor protein. When this information is combined with reports in rats that intra-amniotic administration of ST receptor antibody lowers fetal body weight (29), it is difficult to accept that ST has no role in fetal growth.

Postnatally, serum ST concentrations decline rapidly during the first week of life and continue to decrease, though more gradually, with age, except in pigs, where a 2-week elevation occurs around the time of weaning (19), the time when the hypothalamic-pituitary axis becomes fully functional. The ST receptor levels in pig muscle remain relatively constant with advancing age, whereas those in pig liver increase (149).

IGF-I modulates many of the somatic growth effects of ST postnatally. It also has a role in the developing fetus. However, in cattle (89) and pigs (93), IGF-I levels are low during fetal development and increase postnatally; this is inversely related to growth rate. Thus, if IGF-I is a growth regulator, other elements, such as receptors or binding proteins, must be regulating its activity. The type I IGF receptor has been detected as early as the preimplantation blastocyst stage in cattle (166). Interestingly, bovine IGF-I receptor concentrations differ between muscle types but not between fiber types. In the fetal pig, both IGF-I and type I receptor mRNA are present in muscle at day 60, or midgestation (93). Muscle type I IGF receptor mRNA levels are relatively high compared with levels in other tissues and do not change during the fetal period. Specific binding of IGF-I has been detected in porcine skeletal muscle at day 75 of gestation, increases between days 75 and 90 of gestation coincident with myocyte differentiation, and declines after birth (97). IGF-I binding also appears to differ between muscle types.

In fetal bovine muscle, IGF-II mRNA peaks at around 150 days of gestation and decreases progressively until birth, whereas levels remain constant throughout gestation in the fetal liver (13). IGF-II gene expression in sheep (120) and pigs (93) follows a similar pattern, with peaks at 80 and 60 days of gestation, respectively. Postnatally, serum IGF-II concentrations in pigs gradually rise after birth (92), are elevated just prior to puberty (19), and slowly fall through adulthood. The type II IGF receptor in muscle is barely detectable postnatally, after being highly abundant in porcine fetal muscle tissue (93). Although most physiological effects of IGF-II are thought to be modulated through the type I receptor, there is type II receptor in many fetal bovine tissues throughout gestation; it is differentially and developmentally regulated (127).

IGFs are also regulated by specific IGF binding proteins. The serum concentration of the main carrier protein, IGFBP-3, is low in fetal pigs until late gestation, immediately following the period when IGF-I concentrations in fetal pig serum increase most dramatically (102). These data suggest that the late

gestational rise in IGFBP-3 is due either to an increase in serum IGF-I levels or to development of tissue ST responsiveness. After birth, IGFBP-3 continues to increase gradually into adulthood (93). Skeletal muscle IGFBP-3 mRNA levels, however, are high prenatally and fall after birth (125). Serum concentrations of IGFBP-2 increase throughout gestation in the fetal pig and then decline postnatally (102). Concentrations of IGFBP-1 increase throughout gestation, plateau during the 3 weeks prior to parturition, and peak at birth. The level of IGFBP-1 then declines slightly and remains fairly constant (125). The peak at birth could be related to nutrition because fasting elevates IGFBP-1 levels in serum (104).

Many hormonal and nutritional changes occur from birth through the early postnatal period. The neonate must be able to adjust to a new source of nutrients and maintain a rapid rate of growth. The early postnatal period is characterized by rapid and highly efficient growth. In the neonatal pig, 55% of the energy and 85% of the nitrogen supplied by the milk is retained as piglet weight gain (115), but this efficiency declines with development (39, 136) and parallels the fall in the stimulation of muscle protein synthesis by feeding (39). Insulin can mimic the response of protein synthesis to feeding (174), and anti-insulin antibodies block the response in rats (137). Therefore, insulin is likely an important mediator of muscle protein synthesis, and this high sensitivity and responsiveness to insulin may be crucial for maintaining the high rate of growth in the neonate. One mechanism of growth retardation in progeny of protein-restricted dams may also be associated with insulin. Protein restriction of the dam during gestation and lactation stunts the growth of the progeny's liver and pancreas. The altered organ function may reduce the ability of the pancreas to secrete insulin and decrease the offspring's response to insulin (44).

As animals grow and age, their lean-to-fat ratio usually decreases. Protein accretion rate increases over the growing phase, but at the same time lipid accretion rate increases at an even greater rate (136). The composition of tissue accretion during the growth phase is largely dependent on how an animal partitions its feed rather than due to a mobilization and redistribution of tissue already accrued. Insulin plays an important role in the uptake of nutrients by tissues, and the interaction with the ST/IGF axis is important in the partitioning of nutrients into lean or adipose tissue (14, 54).

POTENTIAL MANIPULATION

Nutritional Manipulation

Although addition of specific nutrients to a diet has less potential to alter body composition than do hormonal or genetic manipulations, nutrients and level of nutrition play a vital role in the distribution of energy and protein sources into

body tissues. Nutritional and hormonal status are tightly linked (Figure 1). Hormonal manipulation of feed intake (e.g. via leptin) affects body composition; conversely, nutrition affects an animal's hormonal balance. Diet and nutritional status are significant regulators of insulin and the ST/IGF axis (5, 106). Nutrition also affects cytokine-insulin and cytokine-ST/IGF interactions (51), and nutritional stress can trigger the immune system, influencing growth and metabolism [reviewed by Elsasser et al (51, 52)]. In addition, nutrition can modify the responses of animals to exogenous hormones (123).

Nutrition also plays a role in gene expression. ST receptor mRNA in the liver (but not muscle) was higher in young pigs fed high versus low levels of energy. This was accompanied by an increase in T_3 and was positively correlated to IGF-I levels and growth rate (37). Chronic protein restriction (irrespective of energy supply) in the growing ewe increased ST-producing cells in the pituitary, apparently by reducing SRIF (128). The effect of protein restriction (constant energy) on suppressing IGF-I is greater in the very young animal, and the effect lessens with age. Protein restriction also decreases hepatic ST receptors in young, but not older, animals (59). Amino acid availability is essential for hepatic IGF-I gene expression. Protein malnutrition not only decreases IGF-I production rate, it also increases its clearance (87).

Not only are hormones and growth factors affected by nutrition, so are their binding proteins. Growth hormone-binding protein is decreased in malnutrition. IGFBPs are also affected by nutrition, but not all respond in the same way. IGFBP-3 is relatively resistant to malnutrition, but IGFBP-1 is acutely regulated by feed intake, increasing during a fast and decreasing after feeding. IGFBP-2 transcription also increases with fasting and malnutrition. Unlike other IGFBPs, IGFBP-2 is responsive to dietary protein intake, increasing with protein restriction (88).

Growth Promotants

Various growth promotants have been studied over the past decade to elucidate their effects on growth performance and carcass composition in both swine and ruminants (Table 1). ST is a potent repartitioning agent in swine, especially in heavier, finishing pigs, whereas the β-agonists are more effective in improving growth performance and carcass composition in ruminant species. However, other than the use of bovine ST in dairy cows and the various steroid implants for ruminants, most of these metabolic modifiers remain unavailable for use in animal production.

Hormones also have a profound effect on how nutrients are utilized. The enhanced insulin sensitivity of whole body amino acid disposal during early postnatal life may be an underlying mechanism contributing to the more efficient use of dietary amino acids for protein accretion in the neonate (172). Therefore,

Table 1 The effects of somatotropin (ST) and other growth promotants on improvements in performance and carcass composition

Metabolic modifier	ADG[a] (%)	Feed:gain ratio (%)	Carcass fat (%)	Carcass protein (%)	Reference
Porcine ST	19	−25	−68	28	55
Porcine ST	16	−32	−51	62	24
Bovine ST	−7	−7	−21	14	111
Bovine ST	5	−12	−12	9	36
Ovine ST	12	−22	−30	36	9
Ractopamine/pigs	8	−11	−15	10	165
Salbutamol/pigs	ND[b]	ND	−11	8	73
Clenbuterol/lambs	24	−19	−27	12	4
Clenbuterol/cattle	ND	ND	−20	13	143
L644,969/cattle	ND	−30	−31	15	109
Zeranol/lambs	12	−9	ND	ND	78
Synovex/cattle	15	−11	ND	4	146
TBA[c] cattle	ND	ND	−15	ND	34
Revalor/cattle	20	−12	ND	34	81

[a]Average daily gain.
[b]ND, No difference.
[c]TBA, Trenbolone acetate.

in the young, growing animal, tissue accretion is regulated such that additional nutrients are most efficiently directed toward growth; however, in the nongrowing adult, tissue mobilization is more highly regulated. At all ages, insulin is released in response to feeding and is crucial in nutrient utilization, affecting both lipid and protein metabolism. Some nutrient partitioning agents act, at least in part, by altering the animal's response to insulin. In growing pigs, ST administration has a large impact on how glucose is partitioned, increasing both blood glucose and insulin concentrations (14). It also has a great impact on how individual tissues utilize glucose. ST decreases whole body sensitivity to insulin (14, 54) and adipose tissue sensitivity to insulin (75), but insulin sensitivity in muscle does not appear to be suppressed by ST (173). Therefore, one mechanism of repartitioning agents is to alter individual tissue responses to homeostatic signals, such as insulin.

Engineering the Genetic Potential

GENETIC ENGINEERING With current embryo microinjection techniques and rapidly evolving cloning strategies, the scientist has great power: to alter one or a few genes in an animal line by generating transgenic animals; or to secure one complete set of genes by generating clones (168). Furthermore, these technologies will likely be interlaced such that a transgenic animal arising from microinjection will be cloned, or a new gene will be inserted into or deleted

from a cell line before its use in a nuclear transfer–based cloning strategy. In fact, a nuclear transfer–based cloning strategy will likely propel a second wave of transgenic livestock research, as did the microinjection-based generation of the first transgenic livestock (70).

TRANSGENIC LIVESTOCK The production of transgenic livestock was first reported in 1985 (70). Since then, transgenic livestock have been produced from approximately 25 gene constructs designed to improve meat production (132, 133). None has yet resulted in commercial application.

A number of hurdles in the production of transgenic livestock for agricultural purposes have been discussed previously (162). The major hurdle is undoubtedly the high cost of producing founder transgenic animals (163). These costs have prevented almost completely the basic research needed to exploit this technology. Some relief may arise from strategies to select for transgenic embryos prior to their transfer to recipient animals. Such a strategy based on use of green fluorescent protein has recently been reported (159). Refinements in livestock cloning strategies may allow selection of transgenic cells before their nuclei are transferred to enucleated eggs. Another hurdle in transgenic livestock production is the time frame required for assessment of a given transgene. Meaningful assessment requires that a number of founder animals be produced because of the variation in transgene expression. Lines of offspring must then be generated for the appropriate production trials. The time from birth of the founder animals to conclusion of growth trials will likely approach 2–3 years for pigs and 4–5 years for cattle. Clearly, the expected phenotypic change due to the transgene will have to be of sufficient magnitude to warrant the time and expense of conducting even preliminary production trials. An unfortunate consequence of the long time frame is the slow rate of progress that can be made by building on experience. And in addition to the somewhat technical aspects of producing and evaluating transgenic animals comes the daunting task of deciding which transgene to microinject.

The use of transgenesis for augmentation of GRF, ST, or IGF-I in pigs has been the major agricultural research target (132, 133). This collection of work has demonstrated the difficulties of using transgenic technology to improve livestock production and has given direction to future research. Transgenic augmentation of the ST/IGF axis was, and is, an obvious target, given the positive results seen with daily injection of ST (14, 54). In general, the transgenic pig projects have yielded desirable effects on growth rate and body composition. However, there were also serious reproductive problems—anestrus in gilts and lack of libido in boars—and other abnormalities such as an increased incidence of gastric ulceration and lameness (133). Given that daily injections of ST are efficacious, the problems encountered with the transgenics have been attributed

to misfunctioning of the control elements included in the transgene, leading to its uncontrolled, lifelong expression. A number of the gene constructs included the promoter/regulatory regions from the metallothionein gene, which is known to be regulatable by dietary intake of zinc. It was presumed that these constructs would be activated by dietary zinc, and that they would be quiescent without supplementation, but they were not. As a result of these findings, a number of laboratories are now conducting research on gene expression systems that can be regulated. The problems encountered with the ST transgenic pigs have also led to a reevaluation of the target gene and have raised the question of whether this technology may be more suited to alteration of specific genes within target tissues rather than as a means to manipulate primary endocrine signals.

INDUCIBLE GENE EXPRESSION Precise temporal and quantitative control of transgene expression will likely be required for the effective use of transgenic technology in animal agriculture. Model regulatory systems currently in development are based on three-part systems containing an externally delivered ligand, a transgene that encodes the ligand receptor, and another transgene that is activated by the ligand-receptor complex and encodes the gene of interest. The first system to be successfully incorporated into transgenic mice (65), and the system that has been most widely studied (153), utilizes tetracycline as the ligand. In this system, the transgene of interest can be negatively regulated by the addition of graded quantities of tetracycline to the water. A second generation of the tetracycline system has also been developed in which the addition of ligand stimulates, rather than represses, the reporter gene (68). A second inducible system uses the insect molting hormone ecdysone as the ligand and a transgene encoding its receptor as the transactivator (114). A third system makes use of a transgene encoding a mutant form of the progesterone receptor that fails to bind progesterone but can bind RU486 (Mifpristone) and other progesterone analogues (164). The mutant receptor was further modified by replacing its DNA-binding domain with the yeast GAL4 DNA-binding domain. This modified receptor will bind to, and direct, the expression of reporter genes containing GAL4-binding sites.

Inducible expression systems have the potential to be of great use in animal agriculture. Tissue-specific expression can be achieved by selecting an appropriate promoter sequence to direct expression of the ligand receptor, and temporal and quantitative expression will be dependent on the supply of an external ligand. However, development of a system that incorporates an acceptable ligand molecule will be important. It is not likely that the consumer will welcome the use of the steroids RU486 or ecdysone, or the use of tetracycline as a growth promotant in transgenic animals.

GENE INHIBITION STRATEGIES Transgenic animal technology is not limited to the addition of functions; it can also be used for gene deletion or inhibition. Gene deletion, targeting, or knockout are strategies currently limited to research with mice. The strategy requires in vitro gene targeting and subsequent selection of modified totipotent, embryonic cell lines (27). Gene targeting is actually replacement of the wild-type target gene with an engineered dysfunctional version and takes place through the process of homologous recombination. To date, the strategy has not been applicable to livestock because of an inability to establish totipotent cell lines. However, with the recent cloning of a sheep from an adult cell that had undergone at least three passages in culture, it seems that gene targeting strategies for livestock will be available in the not-too-distant future (168).

An alternative to gene targeting as a means of inhibiting an endogenous gene makes use of antisense or ribozyme strategies. With these strategies, a transgenic animal is generated in which the transgene encodes a specific mRNA molecule that will hybridize to the endogenous target mRNA. The hybridization prevents translation of the target mRNA, and thus the protein product of the targeted gene is not produced. Translation is prevented by an unknown mechanism that may involve steric hindrance of ribosome binding or enhanced degradation of the endogenous transcript (for review, see 155). Ribozymes are mRNA molecules that contain a catalytic region inserted between two short stretches of antisense regions that confer specificity to the molecule. Ribozymes, which bind and cleave target mRNA, are potentially more effective than antisense molecules that only bind to the target.

Antisense and ribozyme constructs have been used successfully in a number of transgenic mice experiments (155), although much work needs to be done toward understanding the design of effective constructs. Matsumoto et al (101) provided a clear demonstration of the effectiveness of an antisense construct in the inhibition of ST production in transgenic rats. Growth rates in male and female rats that were homozygous for the ST antisense construct were reduced by 50% and 30%, respectively. Plasma ST and pituitary ST mRNA were reduced 30–40%, but besides the dwarfism, no other pathological alterations were observed. The feasibility of ribozyme-mediated gene inhibition in transgenic mice was recently reported by L'Huillier et al (90). They generated lines of transgenic mice containing a ribozyme to bovine α-lactalbumin (α-lac) and then crossbred these mice with a previously generated line of transgenic mice that express high levels of a bovine α-lac transgene. Heterozygous expression of the ribozyme caused greater than a 50% reduction in the level of bovine α-lac mRNA and protein. Thus, gene inhibition by ribozyme or antisense techniques may be a valuable tool in transgenic approaches to changing the genetic potential of livestock.

POTENTIAL TARGET GENES Given that techniques are being developed to change the genetic potential of livestock, the question then becomes which gene or genes should be altered to enhance meat production. Augmentation of a few single-gene traits that would presumably cause a marked beneficial change in body composition have been proposed or have undergone preliminary evaluation (132), the most obvious, and most evaluated, being an elevation of circulating ST concentrations. Other single-gene traits would be variations on this theme, such as an elevation in circulating or muscle-specific IGF-I (32). However, it seems likely that altering the expression of a naturally occurring endogenous gene will require development of a tightly regulated inducible gene system to avoid both overexpression of the gene and compensation by the organism to nullify the transgene's effect.

Another line of research that may have promise is the alteration of metabolic pathways by inserting bacterial genes into animals. Such a strategy was recently used to generate transgenic mice expressing the two glyoxylate cycle enzymes: isocitrate lyase (ICL; EC 4.1.3.1) and malate synthase (MS; EC 4.1.3.2) (147). The glyoxylate cycle permits net conversion of acetate into glucose and is typically found in microorganisms, higher plants, and nematodes. One can only speculate on the impact this system would have on ruminant animals where the majority of dietary carbohydrate is fermented to acetate, butyrate, and propionate, which are absorbed from the rumen. Roughage in particular, as opposed to more costly grain, is fermented primarily to acetate. The ability to utilize acetate for glucose production would likely allow an increase in the amount of forage in the ration without compromising rates of gain obtainable with high grain rations.

Research is also progressing on the insertion of bacterial genes for amino acid biosynthetic enzymes into mammalian systems (8a, 139, 154). The ability to synthesize cysteine was transferred to Chinese hamster ovary cells by transfection with the bacterial genes encoding serine acetyltransferase and O-acetylserine sulphydrylase (154). Subsequently, transgenic mice and sheep incorporating these genes were generated (8a). Protein extracts from various tissues of the transgenic mice contained sufficient quantities of the two enzymes to support moderate levels of cysteine synthesis. Expression levels in transgenic sheep tissues were low.

The biosynthesis of threonine was conferred on mouse 3T3 cells by transfer of four bacterial enzymes: aspartokinase I/homoserine dehydrogenase, aspartic semialdehyde dehydrogenase, homoserine kinase, and threonine synthase (139). These engineered cells were not dependent on threonine for growth and could be maintained in medium that contained no free threonine. This ability to transfer complete bacterial biosynthetic pathways to mammalian systems has great potential for altering the dietary requirement of livestock. Alteration of

other enzyme systems such as desaturases could have a marked impact on meat quality and flavor.

Gene deletion has not yet been performed in any livestock species, but given the recent advances in cloning technology (168), it is likely that such deletions will be performed in the not-too-distant future. An ideal candidate for gene disruption as a means of enhancing muscle mass is myostatin (107). This gene was recently disrupted by gene targeting in mice, with homozygous mutants being viable and fertile. However, at 3 months of age, near the end of the growth period in mice, total body weights of the homozygotes were 34% greater than that of their wild-type littermates. Almost all the increase in body weight was due to a heavier carcass, where weights of individual muscles were approximately twice those of wild-type mice.

SOMATIC CELL ENGINEERING In addition to germ-line modifications, opportunities may develop through advances in nonviral, somatic cell engineering (SCE) or gene therapy. Viral vectors would likely not gain consumer acceptance and are beyond the scope of this review. The nonviral methods for SCE are developing along two paths: those relying on direct introduction of gene constructs to a particular tissue site, and those relying on intravenous delivery of nucleic acids to target tissues. With direct introduction, plasmid DNA is delivered to tissue sites by needle-and-syringe injection (170), jet injection (85), or bombardment with DNA-coated particles (50). These techniques transfect a relatively small number of cells, and thus their usefulness may be limited to specialized applications, such as treatment of localized infection or immunization. Intravenous administration of plasmid DNA, which has the potential to transfect a large number of cells, requires liposome carriers or polylysine-ligand conjugates to protect the DNA from degradation and to enhance transfection efficiency. Naked plasmid DNA is rapidly degraded in serum (30).

The prototypical ligands in polylysine-conjugates are asialoglycoproteins that target the hepatocyte asialoglycoprotein receptor (175). These and other ligands are covalently conjugated to polylysine, a polycation, which binds electrostatically to plasmid DNA. Intravenous administration of such a complex containing a human factor IX cDNA expression plasmid has resulted in relatively high circulating levels (100 ng/ml range) for extended periods (2 to 3 months) (126). This method of SCE is promising, but a recent report describes a substantial immune response to the ligand portion of the complex, which will limit its usefulness for repeated applications (157).

Liposomes have been used primarily as carriers to transfect the lung following intratracheal or intravenous administration (26). Intravenous liposome-DNA complexes also have transfected other tissues, but the efficiency has generally

been low. Their ability to transfect lung following intravenous administration is thought to be related to a "first-pass" clearance of liposomes from the circulation by the lung capillary bed. Recent evidence indicates that liposomes enter the cell via endocytosis (64). The endocytotic process is usually followed by fusion of endosomes with lysosomes and subsequent degradation of endosomal contents. Indeed, part of the effectiveness of liposomes in mediating transfection seems to be due to their ability to disrupt endosomal membranes prior to lysosomal fusion, thus preventing degradation of endosomal contents by lysosomal enzymes.

Research into nonviral SCE is still in its infancy. However, there is already evidence that it will be effective for a relatively short period. This 2- to 3-month period may have limited application in human medicine but could have significant animal agricultural applications given the relatively short life span of animals raised for meat production.

CONCLUSIONS

New horizons in the control of growth and development processes are emerging through advances in genetic engineering. We have reviewed facets of muscle development relative to tissue composition and the influence of nutrients on body composition. We have emphasized that the interactions of nutrition with other regulators of growth and development are probably more important than the direct effect of nutrition on body composition. Future research will likely focus on tissue-specific factors, yielding targeted applications for controlling livestock growth and development patterns and leading to the production of meat with a more desirable and healthful composition than is currently available. Although emerging technologies promise to speed our progress, it is important to continue to gain a more complete understanding of nutritional physiology and to examine its impact on the products of genetic engineering. Increasing our knowledge of nutrition and physiology may also aid in the selection of genes appropriate for genetic engineering technologies.

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