Regulators of Adipocyte Precursor Cells

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ABSTRACT Lean and adipose tissue growth are two of the most important targets for manipulation in commercial livestock. Adipose tissue growth occurs by both hyperplasia and hypertrophy. The processes involved in adipocyte hypertrophy are relatively well understood but much less is known about adipocyte hyperplasia. The mature adipocyte has little capacity for cell division and the hyperplastic capacity of adipose tissue resides in a population of fibroblast-like adipocyte precursor cells. The origin of these cells and the processes involved in their commitment to the adipocyte lineage is not known. Growth factors, in particular the bone morphogenetic proteins (BMP), are likely to be involved in regulating commitment to the adipocyte lineage. *In vitro* studies have shown that once committed to the adipocyte lineage, the proliferation and differentiation of adipocyte precursor cells is regulated by a number of different growth factors. A number of these growth factors are expressed in adipocyte precursor cells *in vitro* and may have an autocrine-paracrine role. Others, such as epidermal growth factor (EGF), are more likely to have an endocrine role. The precise role that each growth factor plays in regulating adipocyte development *in vivo* is poorly understood. The chick is a useful experimental system with which to study the precise function of growth factors in adipocyte development.

(*Key words*: adipogenesis, growth factor, differentiation, cell commitment, chick)

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INTRODUCTION

Consumer demand for lean meat means that producers need to pay much more attention to carcass composition; however, there is an optimum distribution of fat because too lean a carcass can compromise meat quality. Lean and adipose tissue growth represent two of the most commercially important targets for genetic and other manipulation in livestock species. Adipose tissue growth in young animals involves a rapid increase in cell number that involves the proliferation and differentiation of adipocyte precursor cells. An understanding of what regulates these processes is an essential first step in designing methods for the manipulation of adipose tissue growth.

The purpose of this review is to give an introduction to the processes involved in chick adipocyte development and to describe the potential regulatory role of growth factors. This description will deal specifically with white adipocyte development with examples from work in the chick where possible. Brown adipocytes have yet to be described in avian species, but for a review of mammalian brown fat development the reader is referred to Ailhaud *et al.* (1992). The role of circulating hormones (e.g, growth hormone and insulin) and steroids in the regulation of adipocyte development has also been reviewed elsewhere (Ailhaud *et al.*, 1992).

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ORIGIN AND DEVELOPMENT OF THE ADIPOCYTE

Adipose tissue occurs as both discrete depots and within other tissues such as muscle. The chicken, however, has a relatively low intramuscular fat content compared to other commercial species such as cow, sheep, and pig. Adipose tissue depots differ in both their size and time of development. For example, a comparison of the abdominal, neck, and leg depots of the broiler shows that posthatch the abdominal fat pad has a much faster rate of growth. In addition, the abdominal fat pad is barely discernible at hatch, whereas the neck and leg depots are well developed (Butterwith, 1989). There is a correlation in young chicks (up to 7 wk posthatch) between the growth of an adipose depot and cell proliferation as measured by [^{3}H]thymidine incorporation (Butterwith, unpublished observations). This finding suggests that cell proliferation is a major contributor to the growth of these depots in young broilers.

[^{3}H]Thymidine incorporation studies in rats have shown that adipocytes develop by the proliferation and differentiation of cells present in the stromal vascular fraction of adipose tissue and that the mature, fully differentiated adipocyte has no capacity for cell division *in vivo* (Van, 1985). It is possible to isolate these adipocyte precursor cells and culture them *in vitro.*

These cells are able to proliferate and subsequently differentiate into mature adipocytes and provide a good *in vitro* system for identifying possible regulators (Van, 1985). The chick adipocyte precursor system is a particularly good one, as it does not contain any other contaminating cell types, which can be a problem in cell preparations from other species.

A number of preadipocyte cell lines have been developed which, in culture, can be induced to differentiate into mature adipocytes. The 3T3-L1, ob17, and their derivative cell lines are the most commonly used for studying adipocyte differentiation. A subclone of 3T3 mouse fibroblasts is 3T3-L1, which was selected on the basis of its ability to differentiate into adipocytes (Green and Kehinde 1976). The ob17 cell line was derived from dedifferentiated adipocytes from the ob/ob mouse (Negrel *et al.*, 1978). A chick preadipocyte cell line has not yet been developed.

GROWTH FACTOR REGULATION OF PREADIPOCYTE COMMITMENT

There is increasing evidence that growth factors are likely to influence the processes of adipocyte development from the early commitment of a pluripotential cell to the terminal stages of differentiation and the formation of the mature adipocyte. Of particular interest are the bone morphogenetic proteins. Named thus because they induced endochondral bone formation when implanted subcutaneously in rats (Wozney *et al.*, 1988) they have now been shown to have a number of other functions, one of which may be to induce adipocyte commitment. For example, Ahrens *et al.* (1993) have shown that transfection of the pluripotential cell line C3H 10T1/2 with the cDNA for bone morphogenetic protein-4 (BMP-4) causes the cultures to form a dense covering of adipocytes. Although this work involved transfection of the cDNA for BMP-4, it is likely that only a short exposure of the protein to its receptor is required. For example, incubation of C3H 10T1/2 cells with BMP-2 for only 1 h is sufficient to induce commitment to the adipocyte lineage (Wang *et al.*, 1993). This result strongly suggests that the activation of a commitment gene or genes induces a cascade that results in the morphology of an adipocyte sometime later. Unlike BMP-4, addition of BMP-2 to C3H 10T1/2 cells also induces commitment to osteoblasts and chondrocytes as well as adipocytes.

The origin of the stem cell that gives rise to an adipocyte and the processes involved in its commitment are unknown. Unlike muscle, for which a number of commitment genes have been found, the use of subtractive hybridization techniques have not yet identified an adipocyte commitment gene or genes. Colon-Teicher *et al.* (1993) have identified genomic sequences that are capable of committing fibroblasts to the adipocyte lineage, but so far have not been able to demonstrate that these are transcribed into mRNA. Their findings, however provide strong evidence for the existence of adipocyte commitment genes.

Tontonoz *et al.* (1994) have described a lipid activated transcription factor, PPARγ2, which may be involved in adipocyte commitment. Transfection of PPARγ2 into a number of mouse fibroblast cell lines leads to the formation of lipid-containing cells. This process is enhanced by addition of EYTA, a PPAR activator or by cotransfection of C/EBPα an important regulator of terminal adipocyte differentiation. Expression of PPARγ2 is unique to adipose tissue.

Recently, a novel factor named Preadipocyte factor-1 (Pref-1) has been described that may play a role in regulating preadipocyte commitment. Preadipocyte factor-1 is a transmembrane protein, containing six epidermal growth factor-like repeats, which may be a novel growth factor or receptor. Preadipocyte factor-1 is normally only expressed in proliferating preadipocytes and expression is absent from differentiating cells (Smas and Sul, 1993). Constitutive expression of Pref-1 in 3T3-L1 preadipocytes prevents their differentiation into adipocytes (Smas and Sul, 1993). One function for Pref-1 may therefore be to preserve cells in a proliferating undifferentiated state.

It is likely that Pref-1 has a number of other important developmental functions. A number of other genes have been discovered that have complete homology to Pref-1. These genes include the putative homeotic protein dlk, which is expressed in a number of tumors with neuroendocrine features, and pG2, an adrenal specific gene (Helman *et al.*, 1987). Fetal antigen-1 is a circulating peptide that has 100% homology to the extracellular domain of Pref-1 and is probably the secreted form (Jensen *et al.*, 1994). Preadipocyte factor-1 also has significant sequence homology to Notch and Delta, which are thought to be involved in cell fate decisions in *Drosophila* (Rebay *et al.*, 1991).

GROWTH FACTOR REGULATION OF PREADIPOCYTE PROLIFERATION AND DIFFERENTIATION

In vitro studies using adipocyte precursor cell cultures and preadipocyte cell lines have led to the discovery of a number of growth factors that regulate proliferation and differentiation. The insulin-like growth factors (IGF) have been the most extensively studied. Both IGF-I and IGF-II stimulate chick adipocyte precursor proliferation with equal potency (Butterwith and Goddard, 1991). The chicken Type II receptor does not bind IGF-II (Duclos and Goddard, 1990; Clairmont and Czech, 1989; Yang *et al.*, 1991) and IGF-II appears to elicit its response through the Type I receptor. Concentrations of insulin in the microgram range are required to stimulate precursor proliferation and it is therefore likely that this effect is via the IGF-I receptor rather than the insulin receptor.

The action of the IGF-I and IGF-II may be autocrine-paracrine or endocrine. Insulin-like growth factor-I is

expressed in chick adipocyte precursor cells (Burt *et al.*, 1992) and this is also the case in mammalian preadipocytes (Doglio *et al.*, 1987; Gaskins *et al.*, 1990). Expression of IGF-I during differentiation can be stimulated by growth hormone (Doglio *et al.*, 1987; Gaskins *et al.*, 1990). Little is known about the expression of IGF-II in adipose tissue.

Insulin-like growth factor action on adipose tissue is likely to be influenced by the IGF-binding proteins, of which six have now been characterized (Rechler and Brown, 1992). The IGF-binding proteins can either enhance or inhibit the action of IGF-I and IGF-II and this action very much depends on the cell type and particular binding protein under study (for a review see Clemmons, 1992). There is considerable difference in the pattern of IGF-binding proteins produced by preadipocytes from different species and cell lines. Chicken adipocyte precursors secrete IGF-binding proteins of 32.5, 30.5, 27, and 24 kDa and minor ones of 45 and 38.5 kDa (Butterwith *et al.*, 1994). The 27-kDa IGF-binding protein is lost when the cells differentiate (Butterwith *et al.*, 1994). In contrast, rabbit adipocyte precursor cells secrete IGF-binding proteins of 40, 30, and 24 kDa (Nougues *et al.*, 1993), and mouse 3T3-L1 preadipocytes secrete IGF-binding proteins of 46, 34, 30, and 24 kDa (Boney *et al.*, 1994). Peter *et al.* (1993) showed that rat adipocytes expressed only IGF-binding protein-5, whereas the stromal vascular fraction that contains adipocyte precursor cells expressed IGF-binding proteins-2, -3, and -5. Further studies are required to identify the IGF-binding proteins produced by cells from different species and to assess their role in regulating adipocyte development.

A number of fibroblast growth factors (FGF) and their receptors have been identified in the chick (Lee *et al.*, 1989; Pasquale, 1990; Schnurch and Risau, 1991; Zuniga *et al.*, 1993; Halevy *et al.*, 1994). Of these, both FGF-1 and FGF-2 have been shown to stimulate chicken adipocyte precursor proliferation; FGF-2 has the greater potency (Butterwith *et al.*, 1993). The potencies of FGF-1 and -2 are similar in the presence of added heparin, indicating an important role for the extracellular matrix in FGF action (Butterwith *et al.*, 1993). Fibroblast growth factor-2 is expressed in proliferating and differentiated chicken adipocyte precursors in culture (Burt *et al.*, 1992) and in adipose tissue *in vivo* (Burt *et al.*, 1992). An important *in vivo* role of FGF in the regulation of preadipocyte proliferation is suggested by the observation that preadipocytes prepared from obese humans have a greater expression of basic FGF mRNA compared than their lean counterparts (Teichert-Kuliszewska *et al.*, 1992) and have an increased capacity for cell proliferation (Roncari *et al.*, 1986).

There is conflicting evidence as to the role of FGF in the regulation of preadipocyte differentiation. Some studies report an inhibition of differentiation by FGF (Navre and Ringold, 1989; Roncari and Le Blanc, 1990), in contrast to a stimulation (Serrero, 1987; Broad and Ham, 1983), or no effect (Butterwith and Gilroy, 1991). The latter study was performed using chicken adipocyte precursor cells. Interpretation of the data depends on the species and the culture system.

The role of FGF as an autocrine-paracrine or endocrine regulator of adipogenesis is not fully understood, although Roncari has suggested an endocrine role for pituitary-derived FGF or related peptides (Roncari and Le Blanc, 1990). An endocrine function would seem less likely, as neither FGF-1 or -2 are secreted proteins (Abraham *et al.*, 1986; Jaye *et al.*, 1986). Biosynthetic studies have also shown these growth factors to be cell-associated (Klagsbrun, 1989). Other pituitary-derived peptides have been described that stimulate preadipocyte proliferation (Lau *et al.*, 1983) and may have an endocrine role, but these peptides have not yet been fully characterized.

Transforming growth factor-β (TGF-β) stimulates the proliferation (Butterwith and Goddard, 1991) and inhibits the differentiation (Butterwith and Gilroy, 1991) of chicken adipocyte precursors. Expression of mRNA for TGF-β 1 to 3 has been detected in both proliferating and differentiated chicken adipocyte precursor cells (Burt *et al.*, 1992) and TGF-β protein is secreted in a latent form (Butterwith, unpublished data). The TGF-β receptor down-regulation during differentiation may also play a role in regulating preadipocyte differentiation (Serrero and Mills, 1991a) but this is not the case in all cell systems (Torti *et al.*, 1989; Dani *et al.*, 1990; Butterwith and Gilroy, 1991). Whether TGF-β 1 to 3 perform different regulatory functions within adipose tissue is not known.

Epidermal growth factor and TGF-α are potential autocrine-paracrine and endocrine regulators of adipocyte development. Both EGF and TGF-α stimulate the proliferation and inhibit differentiation of chicken adipocyte precursors (Butterwith *et al.*, 1992). Transforming growth factor-α has a greater potency than EGF and this is probably due to the chicken EGF/TGF-α receptor having a higher affinity for TGF-α than does EGF.

It seems likely that EGF has an endocrine role in the regulation of adipogenesis. Epidermal growth factor is not expressed in adipose tissue (Serrero *et al.*, 1993). Treatment of rats with EGF causes a reduction in the number of adipocytes and an increase in the number of adipocyte precursors, leading to a reduction in fat pad weight (Serrero and Mills, 1991b) and depressed levels of plasma EGF are seen in ob/ob mice compared to their control litter mates.

An autocrine-paracrine role for TGF-α seems likely. Transforming growth factor-α protein has been shown to be present in adipose tissue. Crandall *et al.* (1992) and Luetteke *et al.* (1993) have demonstrated expression of TGF-α and EGF receptor in mouse fat pads. Overexpression of TGF-α under control of a metallothionein promoter in transgenic mice leads to a 40 to 80% reduction in epididymal fat and a 50% reduction in total body fat. In this experiment, none of the effects associated with an injection of EGF were seen, such as accelerated eyelid opening and precocious incisor erup-

tion, suggesting that TGF-α was acting in an autocrine-paracrine fashion in these animals.

Platelet-derived growth factor (PDGF) stimulates chick preadipocyte proliferation but its effect on differentiation is not known (Butterwith and Goddard, 1991). Mammalian preadipocyte differentiation is inhibited by addition of PDGF (Hayashi *et al*., 1981; Hauner *et al*., 1995). The expression of PDGF in preadipocytes in culture or in adipose tissue *in vivo* has not been studied.

Mouse 3T3-F442A preadipocytes have been shown to express vascular endothelial cell growth factor (VEGF) in a differentiation-specific manner (Claffey *et al*., 1992). This growth factor is specific for endothelial cells and is likely to play a role in the development of the vasculature within adipose tissue. Previous reports have shown a relationship between the development of blood vessels and the development of adipocytes (Hausman and Kauffman, 1986) and production of VEGF by differentiating adipocytes might provide a signalling mechanism for this. Monobutyrin, a vasoactive lipid secreted by adipocytes, is also likely to be involved (Wilkison *et al*., 1991; Wilkison and Spiegelman, 1993). It is not known whether similar factors exist in the chick.

CONCLUSIONS AND FUTURE PROSPECTS

A number of growth factors have been implicated as important regulators of adipogenesis. In addition they have also been shown to act together in a synergistic fashion (Butterwith and Goddard, 1991; Butterwith *et al*., 1992, 1993). It is likely that adipogenesis *in vivo* is regulated by the coordinated action of a number of growth factors whose expression may vary at different times of development. Having identified a number of potentially important growth factors by *in vitro* studies, the next step is to relate this information to the situation *in vivo*. The chick is a useful model for this, as there are a number of different lines of birds that differ in their rate of fat development. There are also differences in the patterns of development of the different adipose depots within a strain. Measurement of cell proliferation and differentiation *in vivo*, coupled with localization of growth factor-receptor expression in different strains and adipose depots, should contribute significantly to identifying which growth factors are important in the different stages of adipocyte development.

At present, very little is known about adipocyte development in the early embryo. There are no specific markers that can be used to identify preadipocytes *in vivo*. This problem is confounded by only being able to identify adipose tissue when it contains many adipocytes (i.e., terminally differentiated cells). By this stage a number of important developmental stages have been passed. Therefore, the identification of adipocyte commitment genes, etc., that can be used as markers of early adipocyte development is a priority.

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