Adipose*

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Abstract

Adipose, long studied as an energy storage depot and structural tissue, is a key player in maintaining energy homeostasis. Additionally, through its endocrine functions, adipose impacts a wide variety of systems in the body. Adipose is a unique organ in that its mass can vary drastically between individuals, from under 5% of body mass in elite athletes to well over half of body mass in the morbidly obese. Because the range of systems affected by adipose are so broad, it follows that over or under accumulation of this tissue can have vast and important medical consequences. Obesity increases risk for diseases such as type 2 diabetes, high blood pressure, coronary atherosclerosis, gout, cancer, gall bladder disease, sleep apnea, and degenerative arthritis. Lipodystrophy also increases risk for type 2 diabetes. In the US, it is estimated that over 100,000 people die of overweight and obesity related causes each year. 2/3 of the US population is overweight, and 1/3 is obese. This raises many questions: What do we know about fat development? What cell or cells give rise to adipose and what triggers this event? The myriad of roles that adipose tissue plays in the body, as well as the increasing relevance of understanding adipose as it relates to obesity, demonstrate the importance of better understanding this tissue. This chapter aims to provide the reader with an understanding of adipose on a developmental and functional level, as well as present the open questions in the field.

1. Introduction: what is fat?

1.1. Adipose architecture

Fat tissue is composed of a number of cell types: adipocytes, vascular endothelial cells, fibroblasts, and macrophages. Adipose is largely found in areas enriched for loose connective tissue. The major fat depots in mammals are the subcutaneous and intra-abdominal depots. Subcutaneous depots include fat under the skin in primarily the buttocks, thighs, and abdomen. Intra-abdominal fat includes mesenteric, omental, and perirenal fat deposits (see Figure 1). Metabolically active brown adipose in adult humans resides in interscapular, supraclavicular, cerivical, axillary, and paravertebral regions (Nedergaard et al., 2007). Brown fat can also be found in white adipose depots and skeletal muscle in small amounts. In addition to these major depots adipose can be found around organs such as the heart and sex organs, as well as regions as varied as the pads of feet and the bone marrow (Rosen and Spiegelman, 2000).

1.2. Major types of adipose and their transcriptional characteristics

Individual adipocytes fall into two major categories: brown or white. Brown adipocytes are mitochondria rich and lipid poor in comparison with white adipocytes, which contain a single massive lipid filled organelle. White and brown adipocytes play distinct roles in the body: brown fat expends energy, white stores it (Lowell and Flier, 1997). Brown adipocytes express many, but not all, genes in common with white adipocytes (Rosen and MacDougald, 2006). One significant commonality between brown and white adipocytes is the role of the master transcriptional regulator, PPARγ. PPARγ is necessary and sufficient for adipocyte differentiation and is implicated in the transcription of a group of adipogenesis-specific transcripts. Transcriptional targets verified by gel-shift or chromatin immunoprecipitation are listed in Table 1. Further, thousands of genomic loci are bound by PPARγ and coactivators in 3T3-L1 cells, as described in Lefterova et al (Lefterova Mi).

PPARγ is crucial for adipocyte survival: knockdown of PPARγ in mature white or brown adipocytes results in cell death (Gregor and Hotamisligil, 2007). Another transcriptional regulator, C/EBPα, plays a major role in brown and white fat differentiation. C/EBPα activates PPARγ expression, after which the two factors cross regulate each other (Wu et al., 1999). The absence of C/EBPα in white adipose tissue severely blocks development and causes insulin resistance (El-Jack et al., 1999). The absence of C/EBPα in brown adipose delays the expression of a gene crucial for brown adipose function, UCP-1. UCP-1 has been shown to be directly regulated by PPARγ (Sears et al., 1996). Thus, a lack of C/EBPα, which is known to activate PPARγ, hinders downstream PPARγ effects, but does not ablate PPARγ function entirely. This suggests PPARγ is under the control of compensatory mechanisms, though not without altered phenotypic effect, in both brown and white adipose. Simply put, C/EBPα and PPARγ are master regulators of adipogenesis, and will be discussed further throughout this chapter.

1.3. Brown and white fat differences

While major regulators of differentiation are in common between the two cell types, differentiated brown and white adipocytes have significant transcriptional, secretory and morphological differences (Rosen and MacDougald,

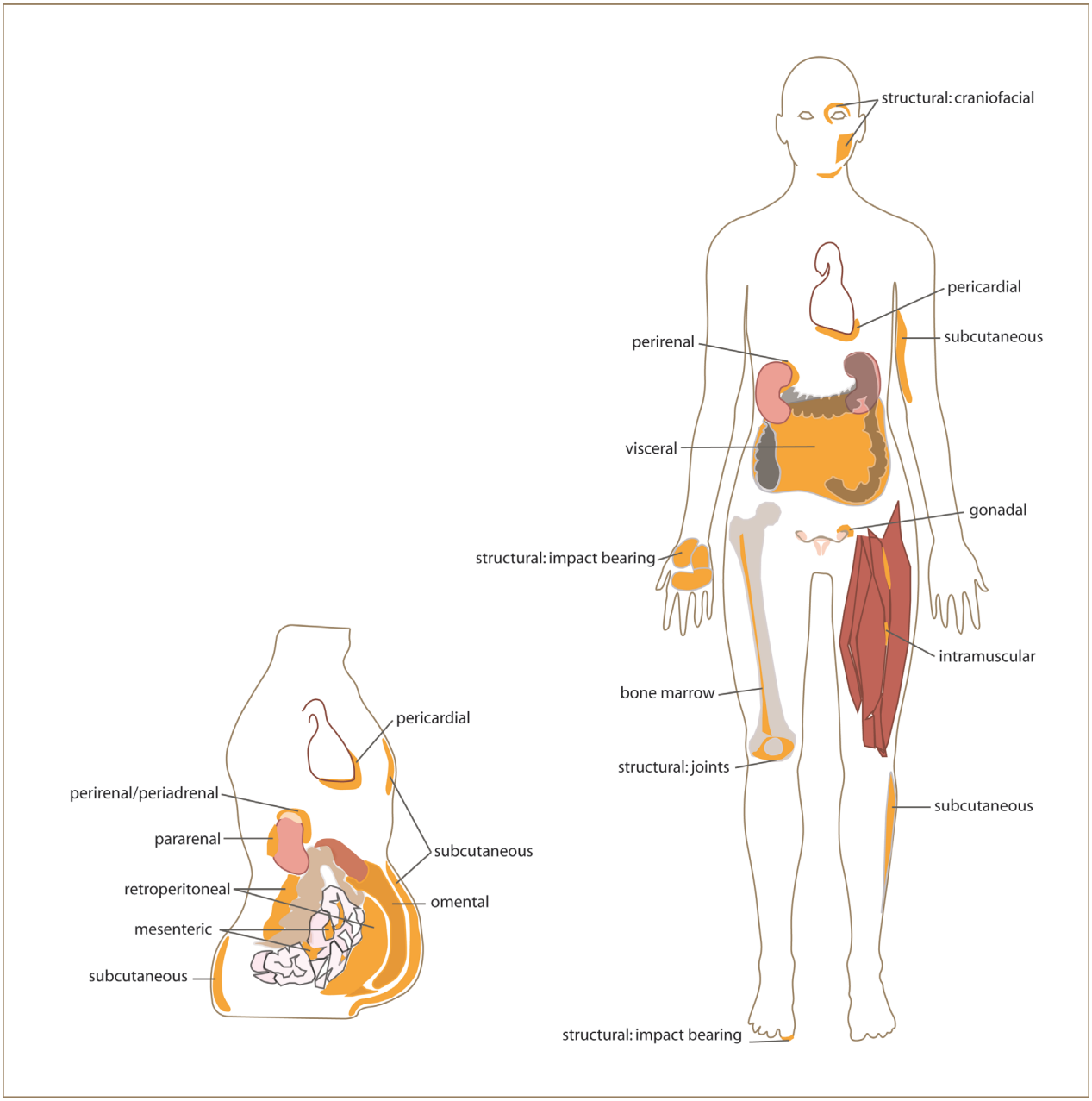

Figure 1. White adipose distribution in the body. White adipose falls under two major classifications: visceral, or surrounding organs, and subcutaneous, under the skin. Fat is distributed widely throughout the body and has different functions and growth properties depending on its location. For example, adipose surrounding sex organs can secrete sex hormones, subcutaneous fat is responsive to energy storage needs and structural fat pads on the feet have not been shown to secrete any factors of interest, nor do they show significant changes in growth. Excessive visceral or gut fat, composed of retroperitoneal fat ("behind the peritoneum"), omental fat (adipose in a sheet of connective tissue hanging as a flap originating at the stomach and draping the intestines), and mesenteric fat (adipose in the sheets of connective tissue holding the intestines in their looping structure), has been shown to be a risk factor for diabetes and cardiovascular disease.

2006). It has been shown that brown versus white determination happens prior to PPARγ action in differentiation: fibroblasts from brown adipose depots differentiate into brown adipocytes and fibroblasts from white adipose depots differentiate into white adipocytes (Klaus, 1997; Seale et al., 2007). Further, brown adipocytes derive from cells expressing a gene previously thought only to be in myogenic precursors, myf5 (Seale et al., 2008). This finding suggests a common origin between brown fat and muscle, but no similar finding was made in white adipocytes.

Table 1. Targets of PPARγ targets and their functions.

PPARγ transcriptional targets	Function
Apolipoprotein-AI, Apolipoprotein-AII, Apolipoprotein-CIII, lipoprotein lipase (Desvergne, 1999)(Nakachi et al., 2008)	triglyceride transport, fatty acid release from chylomicrons to the cell
adiponectin (Nakachi et al., 2008)	hormone that regulates a number of metabolic functions
adipsin (complement factor D; Nakachi et al., 2008)	factor involved in innate immunity
FATP, CD36 (Desvergne, 1999)	fatty acid transfer into the cell
FABP4 (Tontonoz et al., 1994; Desvergne, 1999; Nakachi et al., 2008)	intracellular fatty acid binding and shuttling
ACBP (Neess et al., 2006), Stearoyl-CoA desaturase 1 (Desvergne, 1999)	acyl-CoA binding, desaturation
glucose glycolytic enzymes and GPDH (Zandbergen et al., 2005)	glycerol-3-phosphate production
Acyl-CoA synthase, Malic enzyme (Desvergne, 1999; IJpenberg et al., 1997)	energy utilization in triglyceride synthesis, ATP or NADH generating pathways
phosphoenolpyruvate carboxykinase (Tontonoz et al., 1995)	glycerogenesis
FLJ20920 (Perera et al., 2006)	possible long chain fatty acid ligase
GHITM (Perera et al., 2006)	growth hormone signaling
G0S2 (G0/G1 switch gene 2; Zandbergen et al., 2005), P21 (Morrison and Farmer, 1999)	cell cycle regulation
Perilipin (PLIN; Perera et al., 2006)	lipid droplet coating protein

A recent study of brown and white fat shows that only a few transcription-related genes are uniquely upregulated in brown adipocyte differentiation (Seale et al., 2007). One of these is the zinc finger protein PRDM16. PRDM16 has been shown to interact with and activate PCG-1, a coactivator of PPARγ in brown fat. While it induces increased mitochondrial biogenesis, ectopic PGC-1 expression does not fully recapitulate brown adipocyte phenotype (Puigserver P). In addition to directly activating the expression of PGC-1, the expression of PRDM16 was shown to correlate with increased expression of UCP1, Cidea and Dio2, as well as increased mitochondrial biogenesis, all major hallmarks of brown adipose (Seale et al., 2007). When expressed in white adipose at levels characteristic of brown adipose, PRDM16 can induce brown adipocytes to form in this tissue. Brown adipocytes depleted of PRDM16 lose their 'brown' characteristics but retain adipocyte specific transcripts such as aP2/FABP4, adiponectin, and PPARγ. Thus, PRDM16 is a major regulator of brown adipocyte differentiation and activity. No unique regulator of white adipocyte differentiation has been described.

Recent work by Rodeheffer et al. using known stem cell surface markers defines a set of markers useful in enriching a population of cells from fat tissue in mice likely to be a white adipocyte precursor population(Rodeheffer et al., 2008;). Despite this advance, it remains the case that there are few useful unique markers of white adipocyte precursors or adipocytes available for fat research (Kahn, 2008).

1.4. White adipose depot differences

In addition to white and brown fat serving different purposes in the body, white adipose in different locations in the body can have different functions. Variations in fat distribution in humans are correlated with metabolic disorders (Gesta et al., 2007). For example, aspects of the metabolic syndrome largely correlate with increased visceral fat accumulation. This raises the question of whether adipocytes play a role in the pathology of disease, or if fat accumulation is merely the byproduct of a system gone awry.

The transcriptional profile of adipose tissue varies between depots. Primary preadipocytes from various depots in the body show distinct global transcriptional profile, even after culturing (Tchkonia et al., 2007). These profiles are distinct between depots regardless of sex and adiposity of the donor. Notably, many of the genes that are differentially regulated are implicated in lipid metabolism, adding support to the idea that adipocytes in different depots may exert unique effects on body makeup and metabolism. Transcription profiling in mice and humans has also shown that developmental genes are differentially expressed in distinct depots. In addition, it seems a number of genes have expression correlating with obesity and, more specifically, fat distribution parameters such as waist hip ratio

(Gesta et al., 2006). These insights into transcriptional variability underlying adiposity phenotype provoke interesting questions about the state of obesity and body fat distribution. That obesity is distinguished by a transcriptionally distinct state tells us that the state of this tissue is far more complex than simply "overfed", and that there may be molecular targets not only for perturbing the storage capacity or size of adipose depots, but for a host of obesity comorbid diseases.

2. Fat development

2.1. Early development: mesodermal origin

Adipose tissue is believed to be mesodermal in origin. Mesoderm arises during gastrulation as the middle layer of tissue formed by cells migrating between the endoderm and ectoderm. The primitive mesoderm differentiates into the paraxial, intermediate, and lateral mesoderms. Generally the embryonic mesoderm is thought to give rise to various kinds of muscle including cardiac, all connective tissue, blood vessels and blood, and lymph tissue. The paraxial mesoderm becomes the bone, cartilage, and ligament of the spine and base of the skull, as well as skeletal muscle, dermis and subcutaneous tissue. The intermediate mesoderm gives rise to parts of the urogenital system. The lateral mesoderm gives rise to tissues that form the pericardial, pleural, and peritoneal cavities. [Clinical Anatomy by Systems, Richard S. Snell]. It is thought that adipose tissue in each of the above tissues is derived from the mesoderm in that region (Gesta et al., 2007).

Few studies have been carried out to show the fate of early mesoderm cells. Lineage tracing of paraxial mesoderm shows that interscapular brown fat arises from this tissue (Atit et al., 2006). Interestingly, embryonic stem cells induced to differentiate into neural crest cells can be differentiated into adipocytes. Neural crest is derived from cells that migrate from the lateral margins of the neural tube, and is thus ectoderm. Further, neural crest derivates traced *in vivo* show that in natural development adipocytes around the salivary gland and ears are derived from neural crest progenitors. Other connective tissues and bone in the head and neck have been shown to be of neural crest lineage as well (Billon et al., 2007). Intrascapular, inguinal, and abdominal fat pads have been shown not to be of neural crest lineage (Wrage et al., 2008), leaving the question of developmental origins of the major white fat depots unanswered. That adipose and other connective tissues could arise from multiple germ layers is an intriguing idea.

2.2. Somatic development

2.2.1. Early morphological observations

Work done in the early 20th century showed that developing adipose tissue could be recognized by a proliferation of capillaries in loose connective tissue [Wasserman 1926]. Many reports over the years have noted this phenomenon (Hausman et al., 1980); though the molecular basis for this capillary hyperproliferative relationship to adipogenesis remains unclear. It has been observed in the developing human fetus that blood vessels in the cheek region destined to become a fat pad have a notably thick adventitia of cells of mesenchymal appearance. It seems, when assessed with histological methods, cells proliferate as the vessels grow. This is not true for all developing fat pads. In the mouse epididymal fat pad it has been observed that lipid and glycogen stores in cells form before or during notable capillary growth (Hausman et al., 1980). As assessed by light microscopy, primitive fat organs in the face differentiate between week 14 and 16 of gestation, and begin to form lipid drops at approximately week 23 in humans (Poissonnet et al., 1983). Adipose development in the head and neck occurs first in the body, and not until the fetus is at least 125g in weight. All major depots in the body observed at birth exist by week 23 or approximately 625g (Poissonnet et al., 1984).

The cellular characteristics of differentiating white adipocytes seems to be consistent throughout the body, but the time at which these cells appear varies between depots. Electron microscopy experiments in rats show that, at birth, inguinal adipose tissue is a heterogeneous mix of cells in varying differentiation states that includes mature adipocytes. In contrast, the epididymal fat pad in rats does not contain mature adipocytes until postpartum day seven. Fat pads in early developmental stages in these experiments contained differentiating and mature adipocytes as well as fibroblast-like cells characterized by multiple processes, rich endoplasmic reticulum (ER), and proximity to capillaries. Early differentiating cells contain multiple small lipid droplets in the cytoplasm and evidence of pinocytosis. As cells enlarge, lipid drops coalesce and large pools of glycogen form. It has also been observed that mitochondria go from being spherical to filamentous during differentiation. Mature cells are characterized by a massive lipid drop, reduction in ER and glycogen stores, and a displaced nucleus (Napolitano and

Gagne, 1963). It is likely that these characteristics hold in other true in humans, however limited data is available to support this.

2.2.2. Adult fat growth

A number of studies were performed in the 1960s and 1970s to determine the nature of fat cell division and growth. Until the late 1970s it was widely believed that fat cells in adult animals did not divide, and that adipose depots only grew larger by lipid filling. A 1973 study by Johnson and colleagues concluded that the genetic background of experimental mice was a large determinant of adipose cellularity and size, more so than feeding behavior (Johnson et al., 1973). Later it was shown that inguinal, retroperitoneal, and gonadal adipose depots in rats fed either a high sucrose or high-fat diet increased in size first, and as fat cells achieved a mean size increased cell numbers were observed. These cell numbers endured even after subsequent fasting and weight loss. Fat pad mass increase in this study was independent of "strain, sex, depot, or diet" (Faust et al., 1978). Additionally, tritiated thymidine cell tracing was carried out and showed both that 1) fat cells do not divide (Greenwood and Hirsch, 1974), and 2) that fat cells do divide (Miller Wh, 1984; Pilgrim, 1971). The dynamics of fat cell growth and division are still not entirely clear, and many studies on this topic have subsequently been refuted.

Today, it is accepted that new fat cells are made throughout life. It has been shown that human adult adipocytes have a roughly 10% turnover rate (Spalding et al., 2008). While genetic background may play a role in fat storage it is clear that adipose is a crucial player in a complex network balancing energy homeostasis, and as such, expands to accommodate increased energy intake. Fat growth is variable from depot to depot and has varying growth rates through a human lifespan. On a cellular basis, whether there is a relationship between hypertrophy and hyperproliferation mediated by a maximum cell size or other molecular trigger remains to be definitively resolved (Hausman et al., 2001). Further, the complement of cells that give rise to adipose tissue and under what circumstances remains to be experimentally shown.

2.2.3. Adipose as an endocrine organ

The discovery of leptin in 1994 by Friedman and colleagues is noted as a turning point in the study of adipose as a complex endocrine organ. Transcribed from the obese gene and secreted by many cell types in the body including adipocytes, leptin acts directly on pancreatic β-cells, immune cells, and neurons in the hypothalamic and ventral tegmental areas of the brain to exert effects on satiety, fertility, reproduction, and hemopoesis (Rajala and Scherer, 2003; Zigman and Elmquist, 2003). One downstream effect of leptin signaling is the downregulation of endocannabanoids responsible for increasing appetite. Thus, the effect of leptin on adiposity is mediated through control of appetite and ultimately on energy intake. No studies have shown a direct molecular effect of leptin, or any cytokine, on adipocyte cell division or differentiation *in vivo*. Numerous "adipokines" have been identified since the discovery of leptin. These have effects on systemic inflammation, insulin sensitivity, stress responsivity, reproductive hormone production, and tissue morphogenesis (Hausman et al., 2001; MacDougald and Burant, 2007; Rajala and Scherer, 2003; Rosen and MacDougald, 2006).

3. Adipocyte precursors

3.1. Fat "stem cells" *in vivo*

Traditionally, Mesenchymal stem cells (MSCs) have been thought to be the precursor cell for adipocytes. MSCs are fibroblast-like cells that derive from the embryonic mesoderm. MSCs are defined by their ability to differentiate into a number of tissue types *in vitro*, including adipose, muscle, bone, cartilage, marrow stromal cells, and tendon. MSCs are reported to have been derived from plastic adherent as well as suspension fractions of primary cell cultures (Wan C, 2006). MSC cultures from adipose, called ADMSCs or ADSCs (adipose derived stem cells), are considered heterogenous because attempts to characterize a ADMSC-specific cell surface marker have shown that cells derived from distinct marker sets can have MSC potential (Gomillion and Burg, 2006).

Two recent experiments lay a foundation for further work on adipocyte precursor cells, or preadipocytes. Tang et al. show that a population of cells (that are not lipid-filled adipocytes) are PPARγ positive and are present on the periphery of blood vessels extracted from mouse adipose tissue. These cells differentiate into adipocytes in culture and proliferate and give rise to some adipocytes *in vivo*. These cells are positive for markers of preadipocytes and adipocyte differentiation such as Pref1, GATA3, Smo, and Gli3. These cells were further defined as being in the mural compartment of the vasculature, having smooth muscle-specific and PDGFRβ staining(Tang, 2008).

Simultaneously, Rodeheffer et al. used FACs to isolate a lin$^-$CD29$^+$ CD34$^+$ Sca-1$^+$CD24$^+$ population of cells with an increased capacity for differentiation into adipocytes over a stromal vascular fraction of cells alone. When transplanted into an A-ZIP lipodystrophic mouse these cells reconstitute the epididymal fat pad and correct a host of lipodystrophy-related disease symptoms. Rodeheffer also noted that these cells seem to localize, at least in part, near the vasculature of adipose *in vivo* (Rodeheffer et al., 2008).

While not comprehensive, these two studies provide clues to distinguishing markers and location of cells that eventually give rise to adipocytes.

3.2. Preadipocytes in culture

The physical transition from preadipocyte to mature adipocyte has been characterized *in vitro*. Preadipocytes treated with differentiating agents such as hormones and mitogens (insulin, glutocorticoids, and cAMP inducers) undergo growth arrest, which is followed by clonal expansion. Subsequently, terminal growth arrest correlates with expression of PPARγ and C/EBPα and lipid droplet formation (Gesta et al., 2007; Hausman et al., 2001; Rosen and MacDougald, 2006). While these physical characteristics are observed in most differentiating preadipocyte cell lines, they have not been described experimentally *in vivo*.

There are significant differences between cell lines and *in vivo* adipose tissue. Post-differentiation levels of secreted factors TNFα and leptin in cell culture are far lower than has been measured *in vivo* (Gesta et al., 2007; Rosen and Spiegelman, 2000). Additionally, a major early transcriptional regulator of adipogenesis, KLF4, has significantly higher expression *in vivo* than *in vitro* (Soukas et al., 2001). Adipocyte cell lines are often aneuploid, changing the foundation for possibilities in transcriptional programming during differentiation (Rosen and Spiegelman, 2000). Additionally, primary cultures of stromal vascular cells can differentiate without first undergoing clonal expansion (Entenmann and Hauner, 1996; Rosen and Spiegelman, 2000), casting doubt on the accepted paradigm for events leading to adipocyte differentiation. Notably, the most widely used "preadipocyte" cell line (3T3-L1) was derived from a mouse embryonic fibroblast line, not adipose tissue. More markers of early adipogenesis are much needed, given the differences between differentiated preadipocytes in culture and mature adipocytes *in vivo*, lack of knowledge of upstream events in the PPARγ transcriptional cascade, and the continued challenge of distinguishing cells that are premature, maturing or lipid filled adipocytes.

4. Regulation of differentiation

4.1. Transcriptional cascade

4.1.1. Core transcriptional events of adipogenesis

The transcriptional cascade of events leading to the differentiation of adipocytes has been rigorously investigated. As mentioned above, PPARγ and C/EBPα act in a self-regulatory feedback loop and also activate the expression of genes of terminal adipocyte differentiation. PPARγ heterodimerizes with retinoid X receptor (RXR) to promote expression of its target genes, and RXRα colocalization to PPARγ DNA targets has been shown to be extensive (Lefterova Mi). Studies in 3T3-L1 cells show C/EBPβ and C/EBPδ are expressed before and activate the transcription of the master regulators PPARγ and C/EBPα (Clarke et al., 1997). Notably, cultured cells lacking C/EBPβ/δ have no PPARγ and C/EBPα expression, but in C/EBPβ/δ null mice PPARγ and C/EBPα are expressed, though these animals have reduced adipose tissue (Farmer, 2006). Thus, cell culture experiment findings must be taken in context; *in vivo* systems may be bolstered by redundancy or possess multiple signaling mechanisms to induce differentiation. After the expression of C/EBPβ/δ and before expression of PPARγ in cultured cells undergoing differentiation there is a significant time lag. It is during this time that the expression of zinc-finger transcription factor KLF5 is induced by C/EBPβ and C/EBPδ, and these three factors can all work synergistically to induce PPARγ (Oishi et al., 2005). For a more comprehensive view of the core transcriptional cascade contributing to adipogenesis see Figure 2.

4.1.2. What activates the core transcriptional events of adipogenesis?

There exist several reviews of the transcriptional events leading to adipogenesis that include details of factors that contribute to the transcription of C/EBPβ/δ, as well as additional possible regulators of PPARγ and C/EBPα (Farmer, 2006; Rosen and MacDougald, 2006). These factors include cAMP regulatory element binding protein (CREB), which can activate C/EBPβ expression. Also affecting C/EBPβ expression is Krox20/Egr2, a growth response gene (Chen et al., 2005). Acting in concert with Krox20 is KLF4, a known effector of differentiation and proliferation in a wide

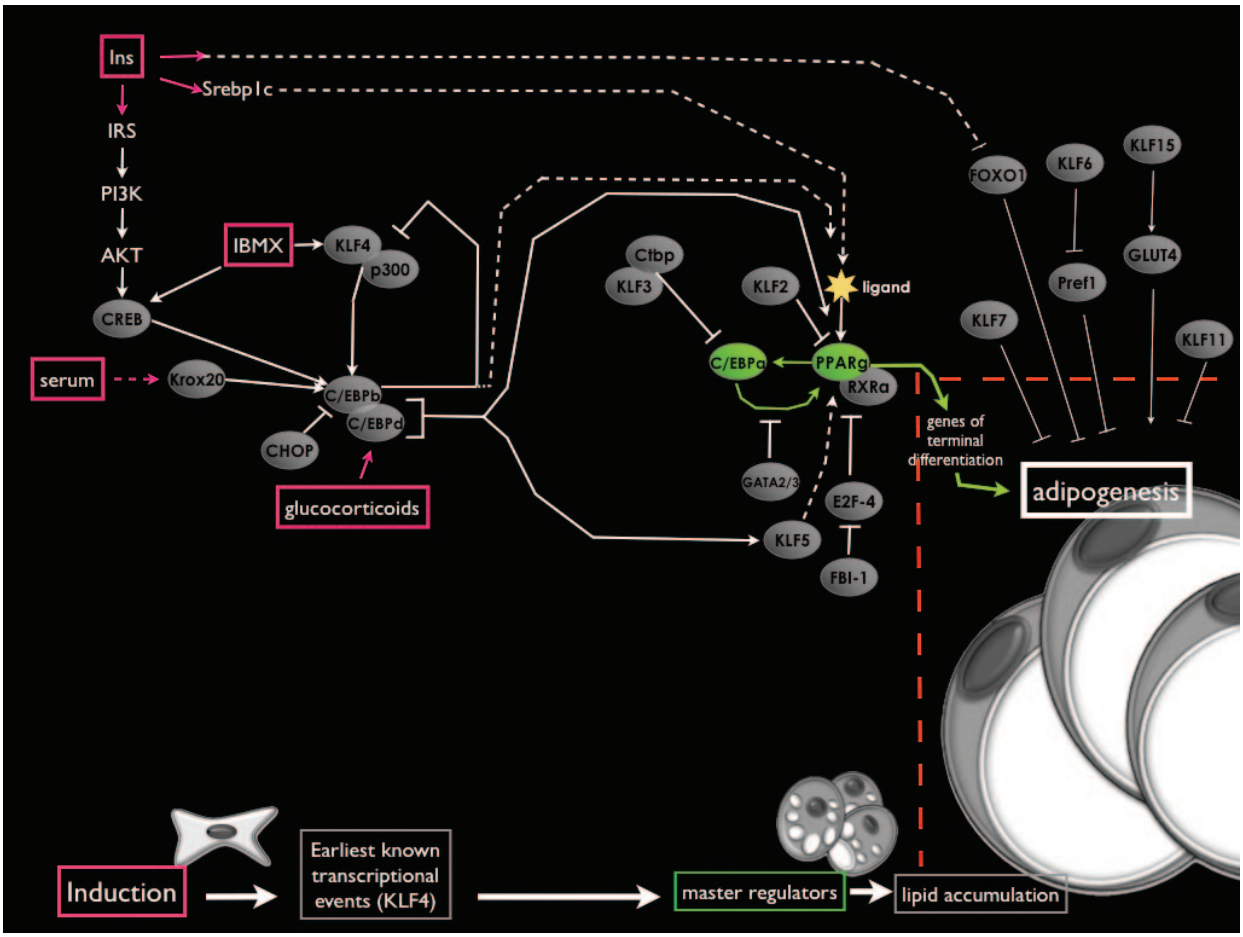

Figure 2. Core transcriptional control of adipogenesis. Cells treated with mitogens and hormones undergo a well-characterized series of transcriptional events. Several signals culminate in C/EBPβ and C/EBPδ activation of PPARγ and C/EBPα transcription. PPARγ and C/EBPα then co-activate each other and a myriad of genes affecting processes of adipocyte differentiation and function.

range of cell types. While KLF4 and KLF5 play a role in upregulation of PPARγ transcription, KLF2 and KLF7 act to inhibit transcription from PPARγ's promoter. Additional members of the KLF family act in promoting adipogenesis: KLF15 promotes the expression of GLUT4, which is responsible for glucose uptake, and KLF6 which inhibits the expression of a major inhibitor of adipogenesis, DLK1/PREF1. The perturbation of any of the above factors has an impact on adipogenesis.

4.1.3. Genetic "toggling" in MSC differentiation

Given the transcriptional timing and sequential action of KLFs at the PPARγ promoter, it is likely that the these factors act in concert to regulate the differentiation state of mesenchymal precursor cells. Another finding that adds to this model is that the PPARγ/RXR heterodimer is in a conformation that can bind corepressors and acts as a negative regulator of transcription when not bound to a ligand (Grimaldi, 2007). This implicates PPARγ as a molecular switch that, when activated by the proper environmental stimulus, acts as a repressor or effector of differentiation state. A "genetic toggle" model appears to be well suited to explaining the intracellular events culminating in adipocyte differentiation.

A myriad of coactivators and corepressors affect adipogenesis in concert with PPARγ and other factors (Rosen and MacDougald, 2006). Mesenchymal stem cells in culture are multipotent cells capable of differentiating into a variety of cell types, including fat. In addition to activation or suppression of differentiation, factors can affect the ultimate fate of mesenchymal precursor cells. An example of a genetic switch in adipocyte differentiation is the interaction of TAZ (a 14-3-3 binding protein) with Runx2 and PPARγ. Runx2 is a major transcriptional regulator of osteoblast differentiation. TAZ acts a coactivator of Runx2 to promote osteocalcin transcription and calcium deposition,

or as a corepressor of PPARγ to block adipogenesis (Hong et al., 2005). Following TAZ-mediated bone differentiation, the repressor MSX2 binds the C/EBPα promoter and suppresses C/EBPα activation of PPARγ (Ichida et al., 2004). Conversely, PPARγ expression downregulates Runx2 expression (Jeon et al., 2003). Notably, siRNA knock-down of PPARγ in embryonic stem cells has been shown to provoke osteoblast differentiation without the addition of additional osteogenic factors (Yamashita et al., 2006). Other molecular switches affecting MSC fate include TIP1 and TIP3 controlling the adipocyte/muscle transition, and HIC5 implicated in adipocyte/epithelial cell fate decisions (Rosen and MacDougald, 2006).

4.2. Epigenetics

Many cellular processes can impact the transcriptional state of a cell that are not strictly genetic, including imprinting, silencing, position effect and DNA and histone modifications. Gaining access to DNA is an important need for the transcriptional machinery in a cell, and a number of proteins and molecules can aid in or block this access, changing the potential for downstream effects.

4.2.1. Is mitosis a requirement for differentiation?

It is widely believed that adipogenesis requires mitosis to reorganize chromatin (Farmer, 2006). Cultured cell lines exposed to differentiating agents undergo one or more rounds of clonal expansion before forming massive lipid droplets. While some primary cultures of human adipose stromal cells do not replicate in culture before differentiating it has been postulated that these cells have already undergone the requisite replications *in vivo*. Specifically, it has been shown that C/EBPβ is transcribed early in differentiation but does not achieve DNA binding capabilities until about 24 hours after hormonal stimulation, which correlates with entry into S phase. Treatment of cells with the MAPK inhibitor U0126 blocks mitotic clonal expansion; cells treated with this inhibitor fail to terminally differentiate (Tang et al., 2003). It should be noted that MAPK signaling has been implicated as having a role in adipogenesis (Rosen and MacDougald, 2006), though if or how this relates to the cell cycle is not completely clear.

4.2.2. DNA modification

Methylation of DNA can be viewed as a mark of transcriptional regulation. DNA methylation can function to repress transcription either by directly blocking protein binding to DNA or by recruiting transcriptional effectors. DNA methylation has also been shown to function as an activator enabling transcription-promoting factor recruitment, though this idea is relatively new and not well studied in adipocytes. Limited information is available in the literature regarding the methylation state of lineage-specific promoters in MSCs. In one study of adipose tissue-derived cells, CpG methylation of adipogenic promoters was highly variable between clonal populations of primary cultures and between donors, and had no correlation with expression levels of the genes investigated (Noer et al., 2006). Another group performed a comparison of bone marrow derived and adipose derived stem cells' methylation state, which showed the promoters of most adipogenic and osteogenic factors were not highly methylated. Expression levels of genes in this experiment correlated with extent of methylation. Inexplicably, PPARγ2 became hypomethylated in bone marrow derived cells with strong osteoblastic differentiation transcription levels (Kang et al., 2007).

4.2.3. Chromatin modification

A possibly more useful mark than DNA methylation for determining the transcriptional state of a cell is covalent chromatin modification, such as histone acetylation or methylation. For a review of modifications of this class see (Kouzarides, 2007). Polycomb group proteins (PcG) are known to be initiators of chromatin modifications as well as transcriptional regulators that recognize these marks. Cells depleted of polycomb, a component of polycomb repressive complexes, show increased expression of genes known to perturb MSC lineage fate, suggesting polycomb is a major regulator of MSC multipotency and cell fate (Bracken et al., 2006).

Several adipocyte-specific genes' promoters in 3T3-L1 preadipocytes have been shown to have H3K4 dimethylation marks on histones prior to terminal differentiation. These marks do not exist in other fibroblast lines. RNA Polymerase II recruitment is associated with these marks, which the authors suggest is indicative of a "adipocyte committed" phenotype (Musri et al., 2007). Globally, HDAC1 and HDAC3 have been shown to play a role in repressing anti-adipogenic genes and allowing the expression of pro-adipogenic genes in concert with many of the molecular switches discussed in section IV A3. Another example of chromatin modifications playing a role in differentiation is the action of TIP proteins. TIP1, whose expression is induced by cell stretching, (pro-muscle) and TIP3 (pro-adipocyte) have histone acetyltransferase (HAT) domains, suggesting a role in recruiting factors that effect chromatin state and

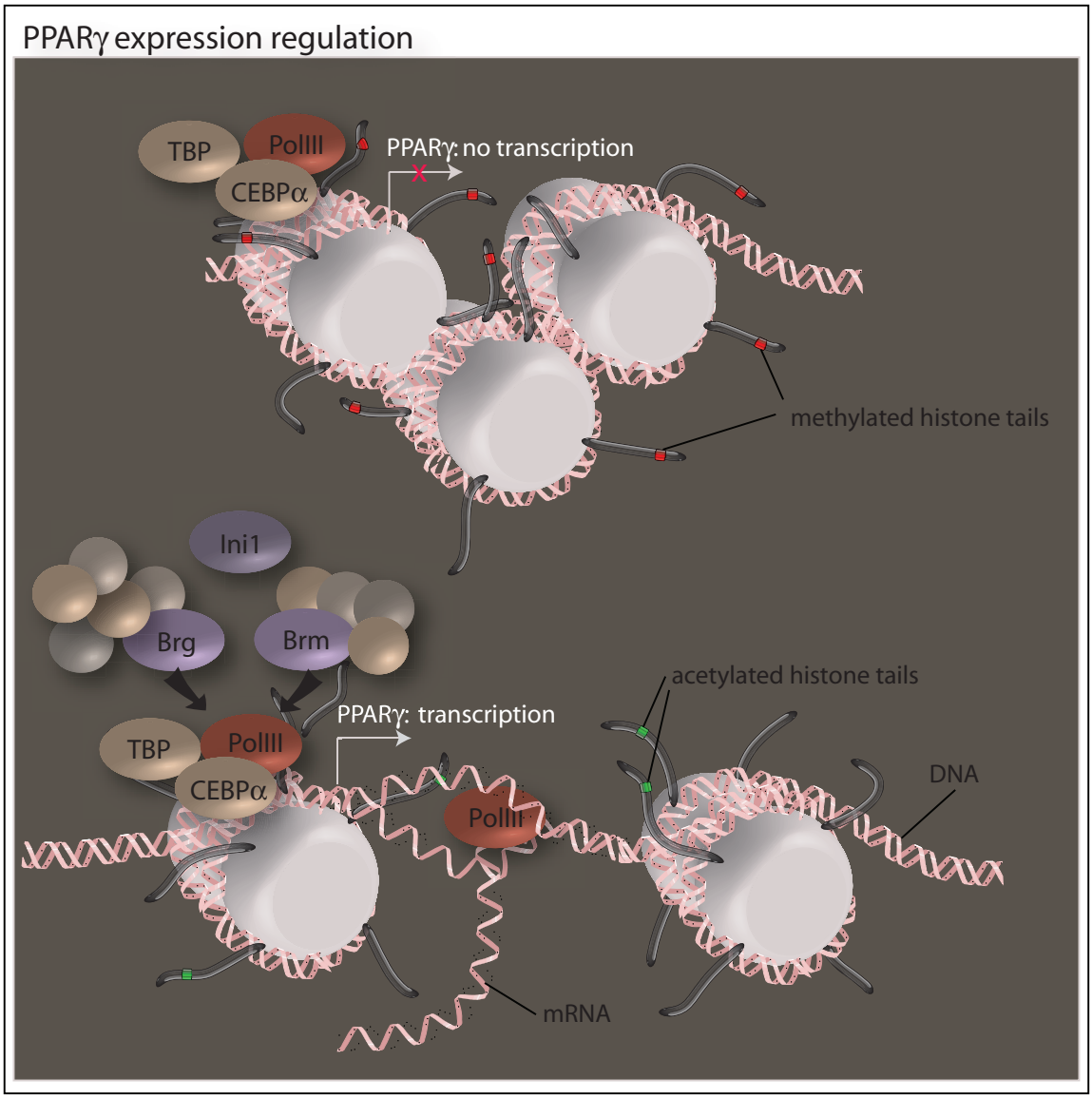

Figure 3. Epigenetics of adipose development. During differentiation a myriad of adipogenic loci are under the influence of chromatin modifying complexes. This figure outlines 2 major points of regulation of adipogenesis. a) Regulation of PPARγ. A number of proteins bind to the PPARγ promoter within a day of induction of differentiation, however no transcription takes place until SWI/SNF subunit Ini1 and complexes with Brg1 and Brm subunits are shown to bind. These complexes remodel the promoter, and transcription is active for several days until the Brg1 and Brm complexes dissociate.

histone modification (Musri et al., 2007). The major regulators of adipogenesis PPARγ and C/EBPα are affected by chromatin modifying proteins (see Figure 3).3 The association or dissociation of HDACs with the promoters of these two master regulators has been shown to correlate with other known steps in the transcriptional cascade leading up to differentiation. (Zuo et al., 2006).

4.3. Signaling

It is likely that the multipotent state of MSCs is maintained by repressive and activating proteins and chromatin. Much is known about events that can push this balance towards terminal differentiation; in fact essentially every major signaling pathway has been implicated in affecting adipogenesis. Early development of adipose is under the control of several conserved developmental genes and their extracellular signaling products. Nodal, BMP, FGF, Hedgehog and Wnt are important for adipogenesis (Gesta et al., 2007) and are also implicated in hESC pluripotency maintenance (Xiao et al., 2006). Additionally, insulin, IGF1 and TGFβ are known to regulate adipogenesis as extracellular signals.

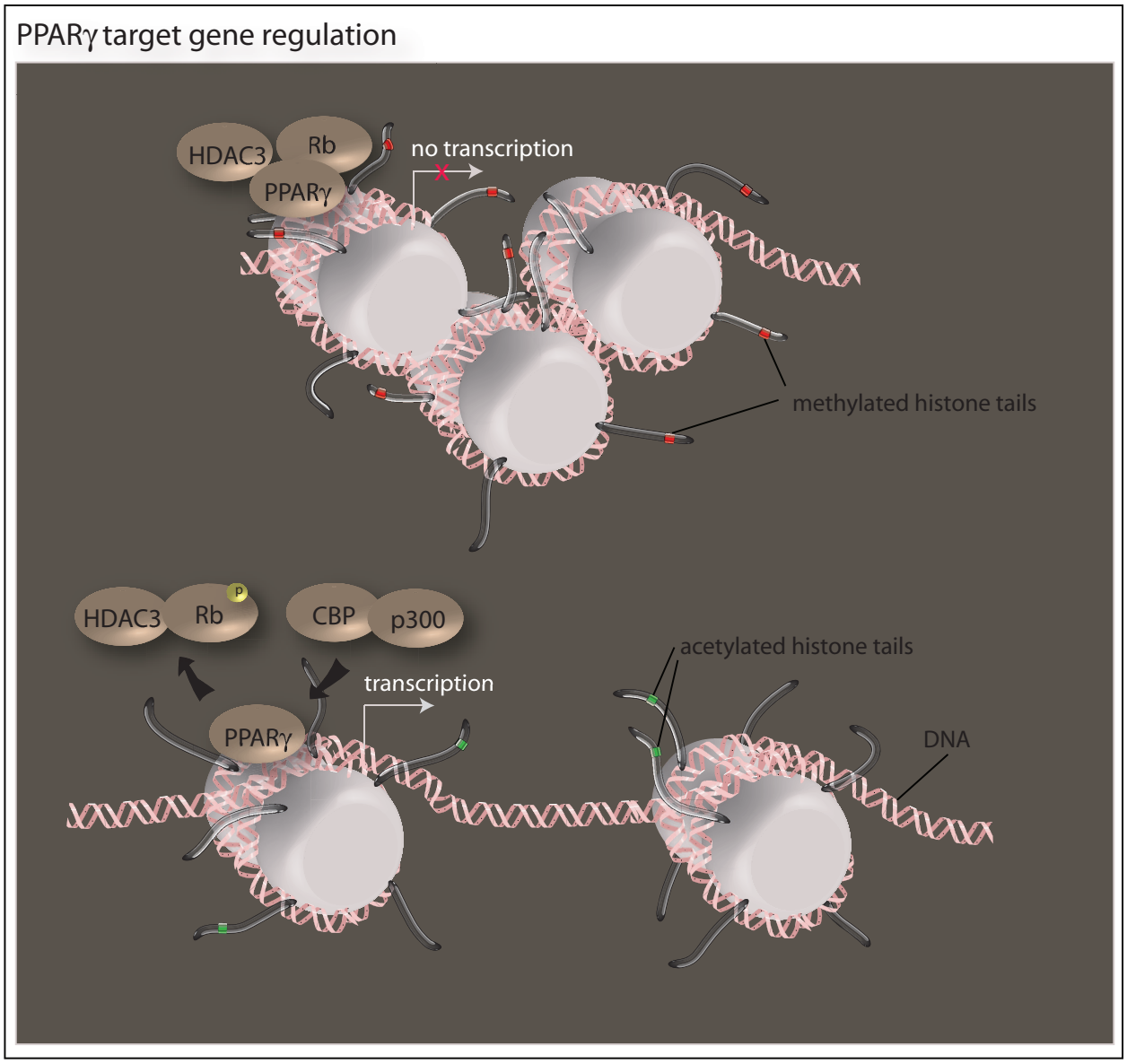

Figure 3. (*Continued*) **Epigenetics of adipose development**. During differentiation a myriad of adipogenic loci are under the influence of chromatin modifying complexes. This figure outlines 2 major points of regulation of adipogenesis. b) PPARγ target regulation. PPARγ acts on a variety of genes implicated in adipocyte differentiation and function. Rb, a factor that binds PPARγ, is known to have a negative effect on PPARγ action in adipogenesis. This is due to its binding deacetylases in its unphosphorylated form that then act on PPARγ's target promoters. In the event that Rb is phosphorylated PPARγ binds acetyltransferases, which then acetylate histones at PPARγ target promoters, allowing transcription to occur.

The pathways these signals activate leading to the eventual adipogenic transcriptional program are not entirely worked out. For a thorough review of what is known see (Rosen and MacDougald, 2006).

Knocking out developmental signaling molecules in mice typically has lethal or deleterious phenotypes as their function is not limited to adipogenesis. *In vitro* and limited *in vivo* studies have shown however that many signaling molecules can exert different effects on differentiation depending on concentration and the complement of other signaling molecules present. This adds a layer of complexity to the problem of deciphering what specific events lead to adipoctye hypertrophy and hyperproliferation *in vivo*.

4.4. Open questions, lines of query to be taken

It is known that PPARγ is a master regulator of adipocyte cell fate, and that many signaling events perturb this fate. It has been observed that the stromal vascular cells in adipose, including mural epithelial pericytes surrounding vasculature, beget mature adipocytes. Several key assumptions in adipogenesis have not been experimentally proven. These include the idea that clonal replication is required for adipogenesis *in vivo*, and that mature adipocytes are terminally differentiated. Many important questions remain in dispute, such as the maximum cell size hypothesis where precursor cells divide and grow when the existing adipocyte population reaches a mean volume. A variety of signaling pathways impact fat depot development, just what signals contribute to the expansion and differentiation of adipocytes has yet to be teased apart. The past year has seen exciting new discoveries shed light on the population of cells that give rise to adipocytes; the elucidation of a single definitive marker for preadipocytes and adipocytes will be a useful tool for describing what the series of events are *in vivo* that lead to a mature fat pad and its growth.

5. Disease and therapeutic use of adipose

5.1. Lipodystrophy

The study of lipodystrophies has helped to gain insights into adipose biology not achieved by other molecular biology approaches. A number of genes have been identified as being a causative factor in familial lipodystrophies, and these genes are involved in processes ranging from lipid metabolism (AGPAT2) to lipid drop morphology (BSCL2 (Szymanski et al., 2007)) to transcriptional regulators of differentiation (PPARγ) and possibly endocytosis or insulin signaling (CAV1 (Ae Kim et al., 2008; Szymanski et al., 2007)).

Lipodystrophies arising from distinct genetic causes have overlapping but distinct phenotypes. For example, mutations in the caveolin-1 (CAV1), AGPAT2, and BSCL2 genes cause Berardinelli Seip congenital lipodystrophy (BSCL). BSCL is characterized by a generalized loss of fat, typically within the first year of life, insulin resistance, nearly undetectable serum levels of adiponectin and leptin, and severe dislipidemia. (Agarwal, 2006); Kim et al., 2008). An extremely rare (with 1 known family) mutation in CAV1 causes a generalized lipodystrophy with reduced circulating leptin and adiponectin, as well as aspects of metabolic syndrome. The CAV1 mutation proband presented with a later onset of adipose loss than AGPAT2 and BSCL2 mutation patients (8 years of age as opposed to at birth), as well as low serum calcium and magnesium. Growth rates differed between the CAV1 patient and BSCL2 or AGPAT2 mutation patients. These minor differences may provide insight into the molecular mechanisms driving BSCL phenotypes with different genetic determinants in humans.

Intriguingly, patients harboring a mutation in PPARγ present with what is known as a familial partial lipodystrophy with onset ranging from puberty to mid-adulthood. These patients have distal extremity and occasional facial adipose abnormalities (Agarwal, 2006). That a mutation in the major transcriptional regulator of adipocyte differentiation would not show phenotypic abnormalities until puberty suggests that multiple adipogenic pathways must function until puberty, and that in healthy adults PPARγ continues to play a role in adipogenesis.

5.2. Proliferating disease

Abnormal adipose hyperproliferation can lead to lipomas and lipomatosis, as well as more serious liposarcomas. A lipoma is a benign soft tissue mass that is typically subcutaneous in location and slow growing. Lipomas are thought to occur in approximately 1% of the population, however the sometimes hereditary condition of acquiring multiple lipomas, lipomatosis, is rare. In some cases of lipomatosis lipomas form in the face and are accompanied by abnormal growth of bone and muscle in the region adjacent to the lipoma. This phenomenon suggests that there could be a MSC specific growth signal in this tissue. There are several distinct characteristic distribution patterns for lipomatosis, for example around the head and neck, or about the trunk and extremities. Benign lipomas are most commonly removed for cosmetic reasons, however many excised lipomas regrow. Familial forms are thought to possibly be autosomal dominant in nature. Liposarcomas are the most common soft tissue malignancy and have a poor prognosis due to difficult detection, as these tumors are typically in deep connective tissues and do not exhibit symptoms until large in size. Exploring what makes lipomas and liposarcomas grow and regrow, as well as their signaling effects to local surrounding tissue, should shed light into normal adipose development, as well as make strides toward treating these disorders.

5.3. Animal models

A number of mice models of aberrant adipose accumulation exist. Several of these recapitulate human disease; for reviews, see (Agarwal AK, 2006; Robinson et al., 2000;). Additionally, an *in silico* analysis of knockout mouse phenotypes predicts that thousands of genes are implicated in weight loss (Reed et al., 2008). Often, lipodystrophies are characterized by components of the metabolic syndrome, which is a predisposing factor for type-2 diabetes. These components include impaired glucose tolerance, elevated serum triglycerides, insulin resistance and fatty liver. Thus, a number of mouse models of reduced adiposity have been used to investigate the pathology of metabolic syndrome and diabetes (Reitman et al., 1999). These include a fat specific dominant negative A-ZIP/F. This protein heterodimerizes with B-ZIP factors crucial to adipogenesis (including C/EBPs). While this mouse strain does not fully recapitulate the metabolic phenotype of type-2 diabetes they have severely reduced white fat and are a useful tool for teasing apart the molecular basis of fat development.

The most famous and widely used mouse model of obesity is the ob-/- mouse. This mouse, deficient in leptin, is obese, and exhibits hallmarks of metabolic disorder. Unfortunately, few humans are obese due to mutations in this gene, and administering leptin to overweight individuals does not seem to have an effect on satiety (leptin functions to signal the 'fed state' of energy reserves to the brain, which responds by suppressing hunger). Several other mutant mice with alterations in the leptin signaling pathway exist, such as db/db (the leptin receptor), and melanocortin mutants. These mice are also obese and display aspects of metabolic syndrome. As expected, many obese human individuals have high circulating levels of leptin, however it seems that the feedback to the brain in these individuals is ineffective. Why this occurs is poorly understood. Despite this mystery, mouse models of obesity still have much to offer in the way of gaining a better understanding of the serious health risks associated with obesity in humans due to the similarities of the components of metabolic syndrome between mice and men.

5.4. Clinical applications, cell based therapies

The potential utility of adipose-derived stem cells is vast. The availability of a large multipotent progenitor cell population has implications for general research, treatment of birth defects or deformities from surgical procedures, and as a treatment for a wide range of diseases.

Autologous transplant is an attractive method for circumventing immune and disease risks associated with non-autologous or engineered tissue therapeutics. Transplantation of fat tissue and cells, however, has significant challenges. Attempts at adipose tissue transplantation in the plastic surgery field have fallen short of the goal of reconstitution of a functional fat pad, as the vascular support of transplanted tissue fails to meet the needs of the transplanted tissue (Gomillion and Burg, 2006). Adipose and adipocytes rarely engraft and proliferate, and instead are reabsorbed. Recent attempts at utilizing patient adipose involve the use of stromal cells isolated from fat depots. These cell populations should include the assumed multipotent progenitor of adipocytes, MSCs. Cells harvested from adipose have been shown to have multilineage potential, opening up the possibility of using these cells to repair such tissues as bone, cartilage and cardiac and skeletal muscle (Fraser et al., 2006; Schaffler and Buchler, 2007).

ADSCs, maintainable in culture, can be directly injected, co-injected with other cell types or small scaffolds, or grown *ex vivo* on specialized scaffolds designed to aid in surgical transplant efficiency (Gomillion and Burg, 2006). Additionally, adipocytes or preadipocytes could be used to introduce genes or gene products into tissues that are damaged or diseased (Morizono et al., 2003; Schaffler and Buchler, 2007).

Many early studies seeking a therapeutic effect from adipose derived cells focused on cell replacement. Reports of transdifferentiation and engraftment in damaged and targeted tissues vary, and have been controversial. Intriguingly, it has been shown that levels of engraftment or differentiation do not always correlate with therapeutic effect. For example, in a cisplatin-induced model of kidney injury, ADSCs injected intraperitoneally in mice did not migrate to tubules, however tubules were significantly less damaged in these mice than in non-ADSC injected controls. It was further found that conditioned medium from cultured stromal cells produced a similar therapeutic effect on the kidney (Bi et al., 2007). Animal models of myocardial infarction, lung injury, and diabetes have all shown therapeutic improvement with MSC therapy(Bi et al., 2007; Lee et al., 2006; Valina et al., 2007). Autologous MSC transplants in human children with osteogenesis imperfecta showed marked improvement of symptoms, however cells did not engraft or persist, and thus did not exert a lasting effect (Horwitz et al., 2002).

It is not known what the molecular mechanism for therapeutic effect of transplanted ADSCs is. Given that, collectively, the above studies suggest that ADSCs are not replacing damaged tissue directly, and that adipose is a crucial endocrine organ in the body, it follows that these adipose-derived cells may be secreting factors that enhance native stem population regeneration, suppress apoptosis or immune response (Bi et al., 2007; Phinney and Prockop, 2007) or perform some other as-of-yet unknown function.

6. Conclusions

Adipose, with its diverse transcriptional and secretory profile, performs a wide range of functions throughout the body. Because of its broad influence on other systems in the body and its extreme plasticity, it follows adipose aberrations have a significant impact on human health. This is seen very clearly in the global obesity epidemic and the resulting host of diseases that accompany overgrowth of fat. Many crucial questions remain unanswered about adipose—what is the full characterization of adipocyte cell of origin, and what perturbs this cell to realize an adipocyte fate? What is the balance of hypertrophy and hyperproliferation that contribute to fat growth? How do brown and white fat in distinct depots act to maintain energy homeostasis, and further, how do cell development characteristics change when this balance is disturbed? What are the intracellular events that lead to the complex fate decision that the presumptive adipocyte precursor must make under a myriad of physiological inputs? Finally, how can we translate lessons learned: how can this knowledge of molecular basis of fate decision impact our ability to realize the potential of adipose derived stem cells in therapies aimed towards regenerating ADMSC and non-ADMSC derived tissues, as a vector for therapeutics, as a production facility for compounds with therapeutic or regenerative potential and as a tool for better understanding adipose overgrowth and obesity? Fortunately, many tools already exist to probe these questions, including a wealth of information pertaining to signaling in adipose development, mouse models, and natural disorders of adipose growth in humans. Thus, given the broad importance of addressing disease and increasing availability of tools and information it is hopeful the coming years will see a surge in the understanding of, and use of, adipose.

7. References

Ae Kim, C., Delepine, M., Boutet, E., El Mourabit, H., Le Lay, S., Meier, M., Nemani, M., Bridel, E., Leite, C.C., and Bertola, D.R., et al. (2008). Association of a homozygous nonsense Caveolin-1 mutation with Berardinelli-Seip Congenital Lipodystrophy. J Clin Endocrinol Metab *0*, 0.

Agarwal AK, G.A. (2006). Genetic disorders of adipose tissue development, differentiation, and death. Annu Rev Genomics Hum Genet *7*, 175–99, 2006;0, 0.

Atit, R., Sgaier, S.K., Mohamed, O.A., Taketo, M.M., Dufort, D., Joyner, A.L., Niswander, L., and Conlon, R.A. (2006). Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. Dev Biol *296*, 164–176.

Bi, B., Schmitt, R., Israilova, M., Nishio, H., and Cantley, L.G. (2007). Stromal cells protect against acute tubular injury via an endocrine effect. J Am Soc Nephrol *18*, 2486–2496.

Billon, N., Iannarelli, P., Monteiro, M.C., Glavieux-Pardanaud, C., Richardson, W.D., Kessaris, N., Dani, C., and Dupin, E. (2007). The generation of adipocytes by the neural crest. Development *134*, 2283–2292.

Bracken, A.P., Dietrich, N., Pasini, D., Hansen, K.H., and Helin, K. (2006). Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev *20*, 1123–1136.

Chen, Z., Torrens, J.I., Anand, A., Spiegelman, B.M., and Friedman, J.M. (2005). Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. Cell Metab *1*, 93–106.

Clarke, S.L., Robinson, C.E., and Gimble, J.M. (1997). CAAT/enhancer binding proteins directly modulate transcription from the peroxisome proliferator-activated receptor gamma 2 promoter. Biochem Biophys Res Commun *240*, 99–103.

El-Jack, A.K., Hamm, J.K., Pilch, P.F., and Farmer, S.R. (1999). Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARgamma and C/EBPalpha. J Biol Chem *274*, 7946–7951.

Entenmann, G., and Hauner, H. (1996). Relationship between replication and differentiation in cultured human adipocyte precursor cells. Am J Physiol *270*, C1011–1016.

Farmer, S.R. (2006). Transcriptional control of adipocyte formation. Cell Metab *4*, 263–273.

Faust, I.M., Johnson, P.R., Stern, J.S., and Hirsch, J. (1978). Diet-induced adipocyte number increase in adult rats: a new model of obesity. Am J Physiol *235*, E279–286.

Fraser, J.K., Wulur, I., Alfonso, Z., and Hedrick, M.H. (2006). Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol *24*, 150–154.

Gesta, S., Bluher, M., Yamamoto, Y., Norris, A.W., Berndt, J., Kralisch, S., Boucher, J., Lewis, C., and Kahn, C.R. (2006). Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci U S A *103*, 6676–6681.

Gesta, S., Tseng, Y.H., and Kahn, C.R. (2007). Developmental origin of fat: tracking obesity to its source. Cell *131*, 242–256.

Gomillion, C.T., and Burg, K.J. (2006). Stem cells and adipose tissue engineering. Biomaterials *27*, 6052–6063.

Greenwood, M.R., and Hirsch, J. (1974). Postnatal development of adipocyte cellularity in the normal rat. J Lipid Res *15*, 474–483.

Gregor, M.F., and Hotamisligil, G.S. (2007). Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. J Lipid Res *48*, 1905–1914.

Grimaldi, P.A. (2007). Peroxisome proliferator-activated receptors as sensors of fatty acids and derivatives. Cell Mol Life Sci *64*, 2459–2464.

Hausman, D.B., DiGirolamo, M., Bartness, T.J., Hausman, G.J., and Martin, R.J. (2001). The biology of white adipocyte proliferation. Obes Rev *2*, 239–254.

Hausman, G.J., Campion, D.R., and Martin, R.J. (1980). Search for the adipocyte precursor cell and factors that promote its differentiation. J Lipid Res *21*, 657–670.

Hong, J.H., Hwang, E.S., McManus, M.T., Amsterdam, A., Tian, Y., Kalmukova, R., Mueller, E., Benjamin, T., Spiegelman, B.M., and Sharp, P.A., et al. (2005). TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science *309*, 1074–1078.

Horwitz, E.M., Gordon, P.L., Koo, W.K., Marx, J.C., Neel, M.D., McNall, R.Y., Muul, L., and Hofmann, T. (2002). Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci USA *99*, 8932–8937.

Ichida, F., Nishimura, R., Hata, K., Matsubara, T., Ikeda, F., Hisada, K., Yatani, H., Cao, X., Komori, T., Yamaguchi, A., and Yoneda, T. (2004). Reciprocal roles of MSX2 in regulation of osteoblast and adipocyte differentiation. J Biol Chem *279*, 34015–34022.

Jeon, M.J., Kim, J.A., Kwon, S.H., Kim, S.W., Park, K.S., Park, S.W., Kim, S.Y., and Shin, C.S. (2003). Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem *278*, 23270–23277.

Johnson, P.R., Stern, J.S., Greenwood, M.R., Zucker, L.M., and Hirsch, J. (1973). Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat. J Nutr *103*, 738–743.

Kahn, C.R. (2008). Medicine. Can we nip obesity in its vascular bud?. Science *322*, 542–543.

Kang, M.I., Kim, H.S., Jung, Y.C., Kim, Y.H., Hong, S.J., Kim, M.K., Baek, K.H., Kim, C.C., and Rhyu, M.G. (2007). Transitional CpG methylation between promoters and retroelements of tissue-specific genes during human mesenchymal cell differentiation. J Cell Biochem *102*, 224–239.

Klaus, S. (1997). Functional differentiation of white and brown adipocytes. Bioessays *19*, 215–223.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell *128*, 693–705.

Lee, B.J., Wang, S.G., Lee, J.C., Jung, J.S., Bae, Y.C., Jeong, H.J., Kim, H.W., and Lorenz, R.R. (2006). The prevention of vocal fold scarring using autologous adipose tissue-derived stromal cells. Cells Tissues Organs *184*, 198–204.

Lefterova, M.I., Zhang, Y., Steger, D.J., Schupp, M., Schug, J., Cristancho, A., Feng, D., Zhuo, D., Stoeckert, C.J. Jr., Liu, X.S., Lazar, M.A. (2008) PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev. 2941–2952.

Lowell, B.B., and Flier, J.S. (1997). Brown adipose tissue, beta 3-adrenergic receptors, and obesity. Annu Rev Med *48*, 307–316.

MacDougald, O.A., and Burant, C.F. (2007). The rapidly expanding family of adipokines. Cell Metab *6*, 159–161.

Miller, W.H. Jr., Faust, I.M., Hirsch, J. (1984) Demonstration of de novo production of adipocytes in adult rats by biochemical and radioautographic techniques. J Lipid Res. 25(4):336–347.

Morizono, K., De Ugarte, D.A., Zhu, M., Zuk, P., Elbarbary, A., Ashjian, P., Benhaim, P., Chen, I.S., and Hedrick, M.H. (2003). Multilineage cells from adipose tissue as gene delivery vehicles. Hum Gene Ther *14*, 59–66.

Musri, M.M., Gomis, R., and Parrizas, M. (2007). Chromatin and chromatin-modifying proteins in adipogenesis. Biochem Cell Biol *85*, 397–410.

Napolitano, L., and Gagne, H.T. (1963). Lipid-depleted white adipose cells: An electronmicroscope study. Anat Rec *147*, 273–293.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab *293*, E444–452.

Noer, A., Sorensen, A.L., Boquest, A.C., and Collas, P. (2006). Stable CpG hypomethylation of adipogenic promoters in freshly isolated, cultured, and differentiated mesenchymal stem cells from adipose tissue. Mol Biol Cell *17*, 3543–3556.

Oishi, Y., Manabe, I., Tobe, K., Tsushima, K., Shindo, T., Fujiu, K., Nishimura, G., Maemura, K., Yamauchi, T., and Kubota, N., et al. (2005). Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. Cell Metab *1*, 27–39.

Phinney, D.G., and Prockop, D.J. (2007). Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair–current views. Stem Cells *25*, 2896–2902.

Pilgrim, C. (1971). DNA synthesis and differentiation in developing white adipose tissue. Dev Biol *26*, 69–76.

Poissonnet, C.M., Burdi, A.R., and Bookstein, F.L. (1983). Growth and development of human adipose tissue during early gestation. Early Hum Dev *8*, 1–11.

Poissonnet, C.M., Burdi, A.R., and Garn, S.M. (1984). The chronology of adipose tissue appearance and distribution in the human fetus. Early Hum Dev *10*, 1–11.

Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M., Spiegelman, B.M. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 829–839.

Rajala, M.W., and Scherer, P.E. (2003). Minireview: The adipocyte–at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology *144*, 3765–3773.

Reed, D.R., Lawler, M.P., and Tordoff, M.G. (2008). Reduced body weight is a common effect of gene knockout in mice. BMC Genet *9*, 4.

Reitman, M.L., Mason, M.M., Moitra, J., Gavrilova, O., Marcus-Samuels, B., Eckhaus, M., and Vinson, C. (1999). Transgenic mice lacking white fat: models for understanding human lipoatrophic diabetes. Ann N Y Acad Sci *892*, 289–296.

Robinson, S.W., Dinulescu, D.M., and Cone, R.D. (2000). Genetic models of obesity and energy balance in the mouse. Annu Rev Genet *34*, 687–745.

Rodeheffer, M.S., Birsoy, K., and Friedman, J.M. (2008). Identification of White Adipocyte Progenitor Cells In Vivo..

Rosen, E.D., and MacDougald, O.A. (2006). Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol *7*, 885–896.

Rosen, E.D., and Spiegelman, B.M. (2000). Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol *16*, 145–171.

Schaffler, A., and Buchler, C. (2007). Concise review: adipose tissue-derived stromal cells–basic and clinical implications for novel cell-based therapies. Stem Cells *25*, 818–827.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H.M., and Erdjument-Bromage, H., et al. (2008). PRDM16 controls a brown fat/skeletal muscle switch. Nature *454*, 961–967.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L.M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B.M. (2007). Transcriptional control of brown fat determination by PRDM16. Cell Metab *6*, 38–54.

Sears, I.B., MacGinnitie, M.A., Kovacs, L.G., and Graves, R.A. (1996). Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. Mol Cell Biol *16*, 3410–3419.

Soukas, A., Socci, N.D., Saatkamp, B.D., Novelli, S., and Friedman, J.M. (2001). Distinct transcriptional profiles of adipogenesis in vivo and in vitro. J Biol Chem *276*, 34167–34174.

Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Naslund, E., and Britton, T., et al. (2008). Dynamics of fat cell turnover in humans. Nature *453*, 783–787.

Szymanski, K.M., Binns, D., Bartz, R., Grishin, N.V., Li, W.P., Agarwal, A.K., Garg, A., Anderson, R.G., and Goodman, J.M. (2007). The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology. Proc Natl Acad Sci USA *104*, 20890–20895.

Tang, Q.Q., Otto, T.C., and Lane, M.D. (2003). Mitotic clonal expansion: a synchronous process required for adipogenesis. Proc Natl Acad Sci USA *100*, 44–49.

Tang, W., Zeve, D., Suh, J.M., Bosnakovski, D., Kyba, M., Hammer, R.E., Tallquist, M.D., Graff, J.M. (2008) White fat progenitor cells reside in the adipose vasculature. Science. 583–586.

Tchkonia, T., Lenburg, M., Thomou, T., Giorgadze, N., Frampton, G., Pirtskhalava, T., Cartwright, A., Cartwright, M., Flanagan, J., and Karagiannides, I., et al. (2007). Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. Am J Physiol Endocrinol Metab *292*, E298–307.

Valina, C., Pinkernell, K., Song, Y.H., Bai, X., Sadat, S., Campeau, R.J., Le Jemtel, T.H., and Alt, E. (2007). Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J *28*, 2667–2677.

Wan, C., He, Q., McCaigue, M., Marsh, D., Li, G. (2006) Nonadherent cell population of human marrow culture is a complementary source of mesenchymal stem cells (MSCs). J Orthop Res. 21–28.

Wrage, P.C., Tran, T., To, K., Keefer, E.W., Ruhn, K.A., Hong, J., Hattangadi, S., Trevino, I., and Tansey, M.G. (2008). The Neuro-Glial Properties of Adipose-Derived Adult Stromal (ADAS) Cells Are Not Regulated by Notch 1 and Are Not Derived from Neural Crest Lineage. PLoS ONE *3*, e1453.

Wu, Z., Rosen, E.D., Brun, R., Hauser, S., Adelmant, G., Troy, A.E., McKeon, C., Darlington, G.J., and Spiegelman, B.M. (1999). Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol Cell *3*, 151–158.

Xiao, L., Yuan, X., and Sharkis, S.J. (2006). Activin A maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. Stem Cells *24*, 1476–1486.

Yamashita, A., Takada, T., Nemoto, K., Yamamoto, G., and Torii, R. (2006). Transient suppression of PPARgamma directed ES cells into an osteoblastic lineage. FEBS Lett *580*, 4121–4125.

Zigman, J.M., and Elmquist, J.K. (2003). Minireview: From anorexia to obesity–the yin and yang of body weight control. Endocrinology *144*, 3749–3756.

Zuo, Y., Qiang, L., and Farmer, S.R. (2006). Activation of CCAAT/enhancer-binding protein (C/EBP) alpha expression by C/EBP beta during adipogenesis requires a peroxisome proliferator-activated receptor-gamma-associated repression of HDAC1 at the C/ebp alpha gene promoter. J Biol Chem *281*, 7960–7967.