A comparative perspective on lipid storage in animals

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Summary

Lipid storage is an evolutionary conserved process that exists in all organisms from simple prokaryotes to humans. In Metazoa, long-term lipid accumulation is restricted to specialized cell types, while a dedicated tissue for lipid storage (adipose tissue) exists only in vertebrates. Excessive lipid accumulation is associated with serious health complications including insulin resistance, type 2 diabetes, cardiovascular diseases and cancer. Thus, significant advances have been made over the last decades to dissect out the molecular and cellular mechanisms involved in adipose tissue formation and maintenance. Our current understanding of adipose tissue development comes from *in vitro* cell culture and mouse models, as well as recent approaches to study lipid storage in genetically tractable lower organisms. This Commentary gives a comparative insight into lipid storage in uni- and multi-cellular organisms with a particular emphasis on vertebrate adipose tissue. We also highlight the molecular mechanisms and nutritional signals that regulate the formation of mammalian adipose tissue.

Key words: Adipocyte stem cell, Adipose tissue, Invertebrate fat, Lipid storage, Nutrients

Introduction

Lipids are a rich source of energy, yielding twice as many calories per gram than do sugars owing to their high-energy bonds (Drewnowski, 1992). In addition to serving as an energy source, lipids are also used as building blocks for membrane biosynthesis, as precursors for the synthesis of other cellular products and as intracellular signaling molecules (Bailey and Dunbar, 1973; Wymann and Schneiter, 2008). Initially evolved as a facultative response to nutrient deprivation in unicellular organisms (Wältermann and Steinbüchel, 2005), lipid storage in the form of triacylglycerol (TAG) is primarily handled by a group of specialized cells, namely adipocytes in vertebrates. Adipocytes constitute the majority of cells in adipose tissue, an active endocrine and immune organ that secretes a plethora of factors (adipokines) that regulate several metabolic processes in distant body tissues. In accordance with the important role the adipose tissue has in the regulation of energy homeostasis, excessive fat accumulation and adipocyte dysfunction have been linked to various health complications, such as insulin resistance, type 2 diabetes, cardiovascular diseases and cancer (Gallagher and LeRoith, 2010). The strong link between obesity and these diseases has greatly stimulated the interest in understanding the molecular mechanisms regulating the formation, maintenance and metabolism of adipose tissue.

In past decades, the development of mammalian cell culture systems that can mimic adipogenesis, i.e. the differentiation of precursor cells into mature fat cells, has allowed the expansion of our knowledge of the molecular events that orchestrate adipocyte formation. Recently, efforts to characterize the identity and localization of adipocyte precursor cells have paved the road to a more complete understanding of adipose tissue formation *in vivo* (Rodeheffer et al., 2008; Tang et al., 2008). In addition, the lipid-accumulation step of adipose formation is highly conserved from prokaryotes to vertebrates. Indeed, genetic screens using genetically tractable invertebrates, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, indicate that many of the central players of lipid storage in lower organisms function similarly to those of higher organisms (Schlegel and Stainier, 2007). Therefore, studying regulation of lipid storage and energy homeostasis in lower metazoans should enable the discovery of new candidate genes and improve our understanding of human metabolic diseases.

In this Commentary, we first provide a concise overview of the lipid accumulation strategies from simple prokaryotes to vertebrates. We subsequently discuss the molecular and cellular mechanisms governing adipose tissue development in vertebrates. Lastly, we highlight the nutritional cues and signaling processes that regulate adipose tissue formation.

Lipid storage – from prokaryotes to mammals

Lipid accumulation as a survival strategy in unicellular organisms

Despite the differences in lipid composition, many eukaryotic and prokaryotic cells are able to intracellularly store lipids. Prokaryotes, for example, store at least one type of lipid in the form of intracytoplasmic inclusions in response to nutrient deprivation or imbalance. Only a subset of prokaryotes (i.e. lactobacilli and enterobacteria) that live in nutrient-rich habitats, such as the gut, lack the ability to accumulate intracellular lipids (Wältermann and Steinbüchel, 2005). Bacterial lipid granules are generally composed of polymeric lipids, such as polyhydroxyalkanoates (PHAs) and polyhydroxyvalerate (PHVs) (Anderson and Dawes, 1990). PHAs are synthesized from soluble hydroxy fatty acid monomers into insoluble high-molecular-mass polymers through successive

elongation reactions catalyzed by a key enzyme only found in bacteria, PHA synthase (Sim et al., 1997). Phasins are the most abundant proteins in these intracellular inclusions, contributing up to 5% of the total cellular protein in bacteria (Steinbuchel et al., 1995; Wieczorek et al., 1995). In prokaryotes, phasin expression and lipid accumulation are tightly coupled and regulated by the cellular concentration of PhaR, a transcriptional repressor that can simultaneously bind to lipids and DNA elements (Maehara et al., 2002; Pötter et al., 2002; York et al., 2002; Yamada et al., 2007). Phasins stabilize lipid granules and prevent their fusion, a role that later in evolution is assumed by lipid droplet (LD)-associated proteins in eukaryotes. The most abundant and conserved eukaryotic LD proteins are those of the PAT family [perilipin, adipose differentiation-related protein (ADRP), tail-interacting protein of 47 kDa (TIP47)] (Miura et al., 2002; Yamaguchi, 2007). PAT-domain-containing proteins can be found early in evolution in organisms as primitive as some fungal species and arthropods (i.e. *D. melanogaster* and *Dictyostelium discoideum*; Wang and St Leger, 2007; Bickel et al., 2009) (Box 1).

Although most prokaryotes store carbon in the form of PHAs, some rare prokaryotes (i.e. Mycobacterium, Rhodococcus and Dietzia) can accumulate lipids in the form of TAGs (Wältermann and Steinbüchel, 2005). Prokaryotic TAGs are synthesized by a bifunctional enzyme that shares no identity to diacylglycerol acyltransferases 1 (DGAT1) and DGAT2, which catalyze the final and the dedicated enzymatic step in TAG synthesis in eukaryotes (Table 1; Box 2) (Alvarez and Steinbüchel, 2002). TAG accumulation in these organisms is induced during the stationary phase upon nutrient restriction (Silva et al., 2010) and is also exploited as a survival strategy in the course of pathogenesis. For example, *Mycobacterium tuberculosis* accumulates lipids during the dormant phase of tuberculosis, a state in which the bacteria can live for long periods of time until the host immune system is weakened (Daniel et al., 2011). Bacteria lacking the ability to accumulate lipids are more sensitive to antibiotic treatment, suggesting that inhibition of lipid accumulation could represent a possible strategy to overcome antibiotic resistance (Baek et al., 2011).

Similar to prokaryotes, simple eukaryotes also store fatty acids under conditions of nutrient limitation, but only in TAG form. Eukaryotic LDs are composed of a hydrophobic core containing TAGs and/or sterol esters that are surrounded by a monolayer of phospholipids and specialized proteins (Walther and Farese, 2012). The composition and size of LDs (0.1 μm in yeast to over 200 μm in white adipocytes) can vary according to the cell type and the external nutritional cues (Table 1) (Walther and Farese, 2009). Even though LDs are generally regarded as independent organelles, it has been shown that they can be connected to the endoplasmic reticulum (ER) through a membrane bridge (Jacquier et al., 2011). This is consistent with the widely accepted hypothesis that LDs are formed in ER and bud from it (Czabany et al., 2007), which is in contrast to the mechanism in prokaryotes, where these droplets appear to be formed from the plasma membrane (Wältermann et al., 2005). Owing to the functional and structural interaction, membrane constituents of LDs and ER can move bidirectionally between both compartments by diffusion (Jacquier et al., 2011). The size of LDs is also dynamic and oscillates during cell growth as a reflection of the balance between TAG synthesis and breakdown (Müllner and Daum, 2004). During proliferation of yeast, TAGs are hydrolyzed by lipases (Tgl3, Tgl4 and Tgl5) and the resulting fatty acids are used for the synthesis of membrane lipids (Athenstaedt and Daum, 2005; Rajakumari et al., 2008). This increase in TAG hydrolysis during cell proliferation is, at least in part, promoted by the activation of *TGL4* by the cell-cycle-related cyclin-dependent kinase 1 (Cdk1, also known as Cdc28) (Kurat et al., 2009). In mammals, the lipases adipose triglyceride lipase (ATGL, also known as PNPLA2), hormone sensitive lipase (HSL, also known as LIPE) and monoglyceride lipase (MGL or MGLL) catalyze the sequential hydrolysis of TAG to diacylglycerol (DAG), monoacylglycerol (MAG), and glycerol and fatty acids, respectively (Zechner et al., 2012). ATGL, which was discovered less than a decade ago (Zimmermann et al., 2004), belongs to the patatin-domain-containing family of proteins that are well conserved throughout evolution. ATGL orthologs are found in essentially all eukaryotic species, including vertebrates, invertebrates, plants and fungi (Zechner et al., 2012). In accordance with such a high degree of conservation, expression of murine ATGL in yeast that are

Box 1. Lipid droplet proteins

Similar to phasins in prokaryotes, eukaryotic LDs contain 'lipid droplet proteins' that protect the core lipids from random degradation. In most but not all yeast species (e.g. *M. anisopliae*) (Wang and St Leger, 2007), homologs of classical metazoan LD proteins, such as PAT family proteins (Perilipin, ADRP and TIP47), have not yet been discovered. However, several comprehensive yeast proteome studies have characterized the LD proteome and identified proteins that are similar to the mammalian counterparts and have roles in lipid metabolism, protein transport and signaling processes (Athenstaedt et al., 1999; Grillitsch et al., 2011). Even though homologs of many LD proteins including seipin, leipin and caveolin exist in worms, no perilipin homolog has been identified in nematode species, suggesting a great structural and functional divergence in these proteins in worms (Mak, 2012). Proteomics studies of fly LDs have also identified LD-associated proteins that are involved in lipid metabolism and transport, intracellular trafficking and RNA metabolism (Beller et al., 2006). Furthermore, insects express two PAT family proteins, lipid storage droplet-1 (Lsd-1) and Lsd-2. Although these proteins are localized on the LD surface, they have both opposing and redundant functions (Bi et al., 2012). Lsd-1 modulates the accessibility of lipases, such as *Drosophila* hormone sensitive lipase (Hsl), to the LD and stabilizes the structure of the LD (Arrese et al., 2008; Beller et al., 2010). Lsd-1 mutant flies are hyperphagic and obese (Beller et al., 2010). Conversely, loss of Lsd-2 results in a decrease in fat mass, whereas ectopic expression of Lsd-2 leads to a dose-dependent increase in TAG storage (Grönke et al., 2003). Reflecting the adaptation of multicellular organisms to nutritional needs, vertebrate LD proteins diversified and are specialized to specific tissues and organs. In addition to perilipin (PLIN1) and adipose differentiation-related protein (ADRP or PLIN2), three additional members of the PAT family of LD proteins, PLIN3–PLIN5 (also known as TIP47, S3-12 and OXPAT, respectively), are present in vertebrates. They are expressed in variety of organs with different temporal and spatial patterns, reflecting their tissue-specific roles (Dalen et al., 2004; Minnaard et al., 2009; Wang and Sztalryd, 2011). In addition to members of the PAT family, the repertoire of LD proteins in vertebrates also includes the cell-death-inducing DNA fragmentation factor-like effector (CIDE) domain family (CIDEA, CIDEB and CIDEC/FSP27), adding more complexity to LD regulation (Wu et al., 2008).

Table 1. Comparison of lipid accumulation from simple prokaryotes to mammals

Taxa	Lipid types	Long-term lipid storage cell or organ	Lipid droplet proteins	Tag synthesis	Lipolysis	Leptin
Bacteria	Polymeric lipids (PHA and PHV); TAG (in Mycobacterium, Rhodococcus and Dietzia)	Unicellular (in response to starvation)	Phasins	PHA synthase; WS/DGAT	HSL and MGL; do not have ATGL	None
Yeast	50% TAG; 50% SE	Unicellular (in response to starvation)	No PAT proteins except for some species (e.g. M. *anisopliae*)	Acyl-CoA dependent (Dga1); acyl-CoA independent (Lro1)	MGL; do not have ATGL/HSL	None
Worms	High TAG, low SE	Specialized cells (i.e. intestinal, skin and hepato-pancreatic cells)	No PAT proteins; other lipid proteins exist (e.g. seipin, leipin and caveolin).	Y53G8B.2	ATGL and HSL; do not have MGL	None
Flies	High TAG, low SE	Fat body	PAT proteins (Lsd-1 and Lsd-2)	Midway	ATGL and HSL; do not have MGL	Upd2 (?)
Fish	TAG and CE (in adipocytes)	Adipose organ and liver	PAT proteins; CIDE domain family proteins	DGAT1 and DGAT2	ATGL, HSL and MGL	Expressed in fat and various other tissues
Amphibia	TAG and CE (in adipocytes)	Adipose organ and liver	PAT proteins; CIDE domain family proteins	DGAT1 and DGAT2	ATGL, HSL and MGL	Expressed in fat and various other tissues
Reptiles	TAG and CE (in adipocytes)	Adipose organ and liver	PAT proteins; CIDE domain family proteins	DGAT1 and DGAT2	ATGL, HSL and MGL	Expressed in fat and various other tissues
Mammals	TAG and CE (in adipocytes); mostly SE (in macrophages)	Adipose organ; subcutaneous adipose tissue	PAT proteins; CIDE domain family proteins	DGAT1 and DGAT2	ATGL, HSL and MGL	Expressed predominantly in white adipose tissue

For references see Boxes 1 and 2. PHA, polyhydroxyalkanoates, PHV, polyhydroxyvalerates; SE, sterol esters; CE, cholesterol esters; TAG, triacylglycerol.

Box 2. Strategies for TAG synthesis

In prokaryotes, the synthesis of TAGs is catalyzed by a bifunctional enzyme, wax ester synthase/diacylglecerol acyltransferase (WS/DGAT). The fusion of the resulting droplets is further regulated by a LD protein called tRNA specific adenosine deaminase (TadA), which has recently been found to be present on *Rhodococcus opacus* LDs (MacEachran et al., 2010). Despite the lack of conservation, WS/DGAT is functional in eukaryotes as expression of WS/DGAT in mutant yeast that is devoid of all TAG biosynthesis enzymes can restore TAG synthesis (Kalscheuer et al., 2004). In animals, DGATs catalyze the final and rate limiting step in TAG synthesis, the covalent addition of a fatty acyl chain to diacylglcerol. In mammals, there are two DGATs, DGAT1 and DGAT2, which account for nearly all TAG synthesis (Harris et al., 2011). However, in yeast, two separate pathways exist for TAG synthesis. As in higher organisms, TAGs can be synthesized by an acyl-CoA-dependent reaction that involves DGATs (*DGA1*). Additionally, yeast can also synthesize TAGs using an acyl-CoA-independent reaction, where phospholipids can act as the acyl donor. The latter reaction is catalyzed by phospholipid/diacylglycerol acyl transferase (PDAT, encoded by yeast *LRO1*), which is homologous to mammalian lecithin cholesterol acyltransferase (LCAT) (Dahlqvist et al., 2000). The exact reason for the evolution of an acyl-CoA-independent pathway in yeast is still unclear, but it might possibly be related to the regulation of membrane lipid composition under different growth conditions (Dahlqvist et al., 2000). Other constituents of LDs in yeast are steryl esters, which are synthesized by the acyl-CoA sterol acyltransferases (ASAT) encoded by *ARE1* and *ARE2* genes (Zweytick et al., 2000). All four lipid storage enzymes (Dga1, Lro1, Are1 and Are2) are surprisingly dispensable for normal growth and survival, as yeast that is devoid of all these four enzymes is viable with no apparent growth defects under normal conditions (Sandager et al., 2002).

deficient in Tgl4 restores their reduced TAG hydrolysis to normal levels (Kurat et al., 2006).

Specification of lipid-storing cell types in metazoa

In invertebrates, specific cell types assume the role of long-term storage of lipids. For example, *C. elegans* accumulate lipids mostly in their intestinal, as well as skin-like, epidermal cells (Nelson et al., 2002; Mullaney and Ashrafi, 2009; O'Rourke et al., 2009). The fat-accumulating organ is much more developed and specialized in arthropods, especially in insects. Here, a specialized organ, often called adipose body or fat body, simultaneously exerts both liver and adipose tissue functions, suggesting that a separation of these metabolic functions to different organs occurred later during the evolution of vertebrates (Arrese and Soulages, 2010). The insect fat body coordinates metamorphosis and reproduction mainly by storing and secreting compounds that regulate developmental processes (Arrese and Soulages, 2010).

The investigation of the mechanisms regulating lipid metabolism in invertebrates, such as *C. elegans* and *D. melanogaster*, has been proven useful for the study of adipocyte biology in higher organisms. Indeed, many conserved proteins discovered in invertebrates have been shown to exert similar functions in mammals. *Adipose* (*adp*) is one such evolutionary conserved gene, encoding a protein with a WD40 domain that regulates chromatin dynamics and gene transcription (Häder et al., 2003). *adp* was initially cloned from obese flies (Doane, 1960), and loss of its function promotes TAG storage in flies and worms, pointing to the crucial role this gene plays in energy homeostasis (Häder et al., 2003; Suh et al., 2007). Similar to in invertebrate models, mice that are heterozygous for *Wdtc1,* the homolog of *adp*, are obese and insulin resistant, which provides further support for a conserved role of this protein in modulating lipid accumulation (Suh et al., 2007).

Among the advantages of using invertebrate models to study lipid metabolism are their convenience and feasibility for genetic and RNA interference (RNAi) screens. Vital dye staining of neutral lipids and buoyancy-based screening methods have been extremely useful to identify genes that affect lipid storage in worms and flies (Ashrafi et al., 2003; Pospisilik et al., 2010; Reis et al., 2010). Such studies identified many genes that are related to lipid metabolism, feeding behavior, nutrient sensing and LD formation. RNAi screens in flies suggest that ~1.5% of the fly genome contributes to LD formation and function (Guo et al., 2008). One gene found in these screens is *FLD1*, the yeast homolog of human seipin (Fei et al., 2008). Seipin loss is associated with congenital lipodystrophy in humans (Magré et al., 2001), a phenotype that might be related to its role in adipogenesis, and LD formation and maintenance (Chen et al., 2009; Chen et al., 2012). Other examples include genes encoding hedgehog, sterol regulatory element binding protein-1 (SREBP1) and CCAAT-enhancer-binding protein (C/EBP), which have functionally similar roles in mammals (McKay et al., 2003; Yang et al., 2006; Pospisilik et al., 2010). Altogether, these results demonstrate that despite seemingly different forms of fat storage, invertebrate models are still instrumental in the identification of pathways that regulate fat accumulation in higher organisms and that many genes that are related to lipid metabolism and storage are functionally conserved throughout evolution.

Starting with primitive vertebrates (jawless vertebrates such as lampreys), lipid-storing cells evolved into a tissue that has distinct functions and a specialized location (Gelman et al., 2009). In fish, amphibian and reptiles, adipose tissue is mainly found in intra-abdominal regions and subcutaneous fat tissue is mostly non-existent. This intra-abdominal location of fat is consistent with the hypothesis that adipose tissue should be at the center of gravity, since it is highly plastic and changes its mass rapidly (Pond, 1992). Furthermore, adipose tissue in lower exothermic animals is usually small because the liver performs the major lipid-storing function. Interestingly, in these animals, the liver secretes many of the mammalian orthologs of the adipokines, such as adiponectin and leptin (Huising et al., 2006; Nishio et al., 2008; Pfundt et al., 2009).

In mammals, adipose tissue has developed to its most evolved and complex form, with a concomitant decrease in hepatic lipid stores. Unlike exothermic vertebrates and invertebrates, mammalian adipose tissue is widely distributed throughout the body, although it is not clear why such a wide fat distribution has been selected by evolution. Even though the presence of fat depots in sub-dermal and subcutaneous areas appear to provide thermal insulation, the observation that there is very little difference in fat distribution between mammals living in tropical versus arctic regions is not concordant with this hypothesis (Pond, 1992). Other selective pressures, such as sexual selection and immune function of adipose tissue, might be important factors driving fat distribution in mammals. Sex hormones indeed affect fat distribution, as evidenced by the increase in subcutaneous adipose tissue in males treated with estrogen (Elbers et al., 1999).

Body fat distribution is a heritable trait in mammals, suggesting that different developmental genetic programs might exist for different fat depots (Bouchard and Tremblay, 1997; Nelson et al., 2000; Baker et al., 2005). Indeed, each adipose depot has a unique gene expression signature during development (Linder et al., 2004; Hishikawa et al., 2005). These signatures are retained when progenitors from different depots are cultured *in vitro*, suggesting an intrinsic program rather than a niche-dependent effect (Gesta et al., 2006; Macotela et al., 2012). For example, patterning genes, including a number of homeobox genes (*Hoxa5*, *Hoxc8* and *Hoxc9*) and *Tbx15* have roles in the patterning of the adipose depot specific program (Gesta et al., 2006). Furthermore, different fat depots respond differently to nutritional status. In obesity, subcutaneous adipose tissue expands through hyperplasia, and adipocyte progenitors are more abundant, in contrast to visceral fat, which grows mainly by hypertrophy (Joe et al., 2009). Taken together, these findings suggest that distinct molecular and cellular programs have evolved to determine the fat distribution in mammals.

Adipose tissue as an active endocrine and immune organ

In addition to its energy-storing role, adipose tissue is an active endocrine and immune organ. Adipocytes secrete a number of adipokines that by acting on the central nervous system and peripheral tissues regulate adiposity, glucose homeostasis, food intake, blood pressure, fibrinolysis, inflammation, lipid metabolism and angiogenesis (Alvarez-Llamas et al., 2007). Besides proteins, adipocytes also secrete lipids (lipokines) that can communicate with distant organs to maintain systemic metabolic homeostasis (Cao et al., 2008).

Leptin is a major adipokine that is primarily produced by mature fat cells and whose expression is highly correlated with the degree of adiposity (Maffei et al., 1995; Birsoy et al., 2008b). Increase in fat mass in mammals causes an increase in the amount of leptin, which provides an afferent signal to the hypothalamus to decrease food intake and increase energy expenditure, thus keeping the body weight stable (Friedman and Halaas, 1998). Even though leptin is also expressed in other vertebrates, its adipose-specific expression and primary role as the pivotal regulator of energy homeostasis appears to be specific to mammals (Table 1). Indeed, in contrast to its adipose-restricted expression in mammals, leptin is expressed in several tissues in fish, including in liver, the biliary system and intestine (Pfundt et al., 2009; Russo et al., 2011; Tinoco et al., 2012). In addition, the anorexigenic effect of leptin appears to be only partially conserved in frogs, rainbow trout, carp and lizards (Niewiarowski et al., 2000; Crespi and Denver, 2006; Murashita et al., 2008; Li et al., 2010). Recombinant leptin also affects metamorphosis, suggesting a pleiotropic role for leptin in the early development of exothermic vertebrates (Crespi and Denver, 2006). Analogous to the endocrine function of adipose tissue, a similar energy homeostasis circuit is believed to be present in lower organisms. Recently, Unpaired 2 (Upd2), a cytokine produced by fat body, has been shown to exert leptin-like effects in flies through GABAergic neurons (Rajan and Perrimon, 2012). Neuropeptide Y, dopamine and serotonin are all present in flies and worms, and modulate feeding behaviors, further confirming the existence of an energy homeostasis circuitry in lower organisms (de Bono and Bargmann, 1998; Srinivasan et al., 2008; Hong et al., 2012).

Adipose tissue also acts as a systemic immune organ and displays significant intrinsic inflammatory properties. Adipocytes react to infections through the activation of multiple inflammatory signal transduction cascades, and the secretion of potent inflammatory cytokines, such as interleukin 6 (IL6) and tumor necrosis factor α (TNFα) (Hotamisligil et al., 1993; Wellen and Hotamisligil, 2003). Interestingly, either bacterial infections

or exposure to lipopolysaccharide (LPS) and/or cytokines can promote adipose tissue lipolysis and reduce TAG uptake by adipocytes (Feingold et al., 1994; Penfornis and Marette, 2005; Zu et al., 2009). Such a lipolytic response to bacterial infections is also present in flies (Dionne et al., 2006). The rise in circulating levels of lipids following infection is thought to support the immune system by facilitating energy production and by neutralizing infectious agents. The intricate balance between metabolic and immune pathways is deregulated in obesity, as an increase in adipose tissue mass results in systemic inflammation and insulin resistance (Hotamisligil, 2010). Adipose tissue inflammation in obesity mimics the 'danger signals' that arise from bacterial infections and this condition promotes the secretion of several cytokines that produce an immune reaction known to cause hyperglycemia and hypertriglyceridemia (Wellen and Hotamisligil, 2003). This immune function of adipose tissue is reminiscent of the immune properties of insect fat body, which expresses the pathogen pattern recognition Toll-like receptors (TLRs) that are required to induce the secretion of antimicrobial peptides upon exposure to microorganisms (Manfruelli et al., 1999; Jang et al., 2006).

Fat tissue development and nutritional regulation

Formation of a fat cell in vertebrates

Adipocyte formation occurs through two stages: (1) the commitment of stem cells into adipocyte precursors and (2) the terminal differentiation of preadipocytes into mature fat cells. Most of our knowledge of the formation of vertebrate fat cells comes from *in vitro* studies using embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and 3T3L1 fibroblasts (preadipocytes). Additionally, differentiation of fish and carp preadipocytes can be induced with a similar generic differentiation cocktail to that used for mammalian preadipocyte differentiation (Vegusdal et al., 2003; Li, 2012) Although these *in vitro* models do not completely recapitulate *in vivo* adipogenesis, these systems have allowed the identification of key regulators of the adipocyte differentiation process such as bone morphogenetic protein (BMP) 2 and 4 (Bowers et al., 2006; Huang et al., 2009), Wnt (Ross et al., 2000; Bennett et al., 2002), transforming growth factor β (TGFβ) (Ignotz and Massagué, 1985), fibroblast growth factor (FGF) 1 and 2 (Widberg et al., 2009; Xiao et al., 2010) and retinoblastoma protein (Rb) (Calo et al., 2010) in the early steps of adipocyte commitment (Fig. 1A;

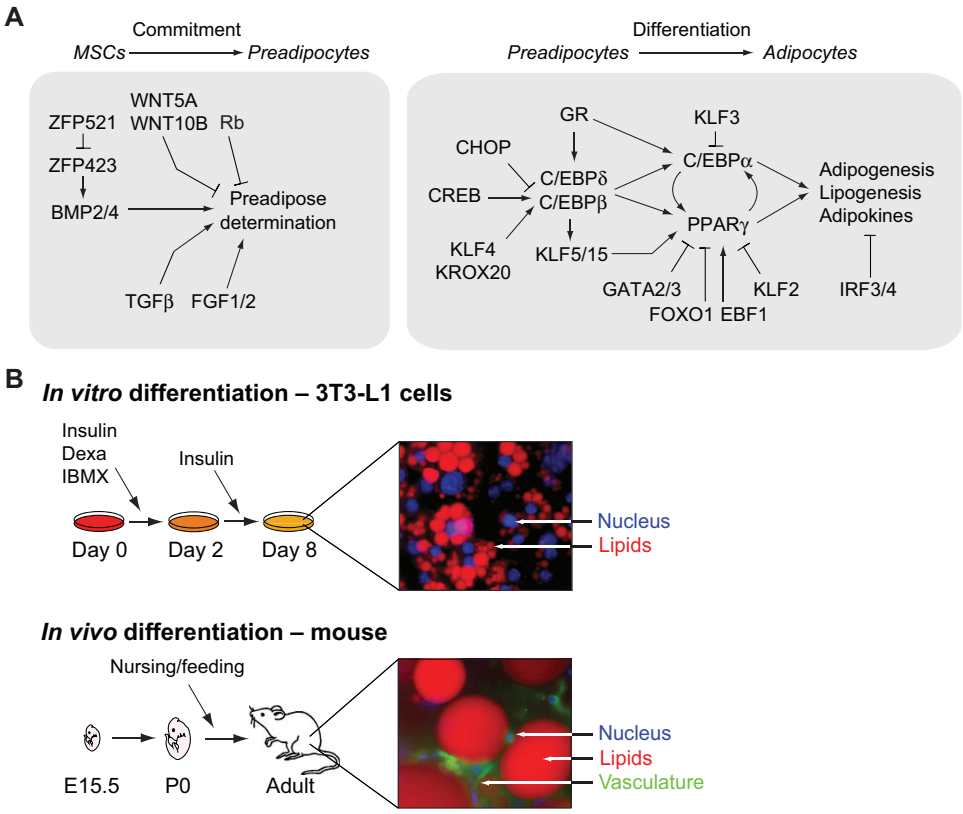

Fig. 1. Overview of the transcriptional cascade regulating the commitment and the differentiation of adipocytes. (**A**) The commitment and the differentiation of mesenchymal stem cells (MSCs) into mature adipocytes follows a well-orchestrated transcriptional program. Although the molecular mechanisms regulating the early events in preadipocyte determination are not perfectly elucidated, key players have been identified. BMP2 and BMP4, Wnt and TGFβ signaling represent some of the pathways involved in the commitment of the MSCs to the adipocyte lineage. The transcriptional events that culminate into the activation of PPARγ and the terminal differentiation of adipocytes are well defined, as presented here. GR, glucocorticoid receptor; CHOP, C/EBP-homologous protein (also known as DDIT3). (**B**) The preadipocyte cell line 3T3-L1 has been extensively used for the study of adipogenesis *in vitro*. At 2 days post-confluence, 3T3-L1 cells are treated with a differentiation cocktail containing insulin, dexamethasone (Dexa) and 3-isobutyl-1-methylxanthine (IBMX), required to activate PPARγ, before the medium is changed and supplemented only with insulin for the rest of the protocol. Upon differentiation, 3T3-L1 cells mostly accumulate lipids in multilocular LDs. *In vivo*, mice are born with no apparent adipose tissue but with committed adipocytes that do not contain any triglycerides. These 'ghost' adipocytes start to accumulate lipids when the animal is exposed to maternal milk and food. In contrast to differentiated 3T3-L1 cells, adipocytes isolated from mouse store triglycerides in a large and unique LD.

Box 3). These models further led to the identification of peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPs as the main drivers of the terminal differentiation process (Rosen and MacDougald, 2006).

PPARγ is a nuclear receptor that has been identified as the master regulator of adipogenesis in vertebrates (Tontonoz et al., 1994). PPARγ is massively induced during adipose differentiation, and constitutive PPARγ expression has been shown to be sufficient to induce adipose differentiation and lipid accumulation in fibroblasts (Tontonoz et al., 1994). Upon ligand binding, PPARγ induces adipogenesis by promoting the expression of key transcriptional regulators of the adipogenic cascade, and by directly inducing the expression of several genes that regulate lipid uptake, synthesis and storage (Gesta et al., 2007). Although different compounds, including fatty acids and prostaglandins, have been suggested to activate PPARγ (Nosjean and Boutin, 2002), bona fide endogenous ligands for PPARγ have not yet been identified. However, there are a number of known synthetic ligands for PPARγ, the best known being the anti-diabetic agents thiazolidinediones (TZDs) (Harris and Kletzien, 1994; Spiegelman, 1998). These drugs have been shown to be efficient in reducing glycemia and triglyceridemia in humans and rodents, in part by reducing ectopic fat storage through shunting lipids to adipose tissue and by promoting the secretion of adiponectin, an insulin-sensitizing adipokine (Tontonoz and Spiegelman, 2008). PPARγ appeared early in the evolution of the metazoan phyla as the result of two waves of gene duplication, the first wave happening before the arthropod–vertebrate divergence (the appearance of PPAR ancestors), whereas the second wave was vertebrate specific (the appearance of PPAR isotypes) (Escriva et al., 2000). Even though no PPARγ homolog has yet been identified in lower organisms, nuclear hormone receptors with similar roles to PPARs have also been discovered in worms and flies. In *C. elegans*, it has been shown that one of these factors, NHR-49, is a key regulator in fat storage and that it has similar biological activity to vertebrate PPARα (Van Gilst et al., 2005). Similarly, *D. melanogaster* has the orphan receptors E75 and E78 that have distant homology to PPARs (King-Jones and Thummel, 2005).

C/EBPs are crucial regulators of development not only in adipocytes but also in a variety of cell types (Nerlov, 2007). C/EBPβ is one of the earliest regulators of adipogenesis, and its expression is upregulated by an early transcriptional and signaling network, which includes of cAMP-response element protein (CREB), Kruppel-like factor 4 (KLF4), and early growth response protein 2 (EGR2, also known as KROX20) (Reusch et al., 2000; Chen et al., 2005; Birsoy et al., 2008a). Induction of C/EBPβ expression further activates the expression of target genes including *CEBPA* (encoding C/EBPα) and *PPARG* (encoding PPARγ). The C/EBP family is composed of six transcription factors that share functional and structural similarity. These similarities suggest an evolutionary history of genetic duplications with subsequent pressure to diversify (Lekstrom-Himes and Xanthopoulos, 1998). C/EBP orthologs are found in eukaryotes from the animal kingdom in organisms as diverse as the Anthozoa (*Nematostella vectensis*), the Crustacea (*Daphnia pulex*), the insects (*D. melanogaster and Apis mellifera*, respectively), vertebrates and in all mammals examined. Other transcription factors in the adipogenic transcriptional cascade have been reviewed elsewhere (Cristancho and Lazar, 2011; Lowe et al., 2011) and include the Kruppel-like family of transcription factors (KLFs) (Mori et al., 2005; Oishi et al., 2005; Sue et al., 2008), glucocorticoid receptors (Yeh et al., 1995), interferon regulatory factors (IRFs) (Eguchi et al., 2008), GATA transcription factors (Tong et al., 2000; Tong et al., 2003), forkhead transcription factors (Nakae et al., 2003) and early B-cell factor (EBF) 1 and 2 (Jimenez et al., 2007).

Box 3. Can *in vitro* adipogenesis recapitulate *in vivo* processes?

Most of our knowledge regarding the processes that govern adipocyte differentiation has been generated through the use of established immortal preadipocyte cell lines (Lefterova and Lazar, 2009). Even though these *in vitro* models are crucial for the identification of the major regulators of adipose tissue development and maintenance, it is difficult to know to what extent these systems could indeed recapitulate the development of fat cells *in vivo*. Several studies provide evidence that differentiation of adipocytes in cell culture recapitulates many of the molecular and cellular properties of the functional adipocytes *in vivo* (Green and Kehinde, 1979; Mandrup et al., 1997; Birsoy et al., 2011). Nevertheless, cell-line-based systems lack several features of adipose tissue, such as the interaction of the fat cells with the extracellular matrix, the cellular geometry and the mechanical stress (Chun et al., 2006; Kilian et al., 2010). These differences could explain why adipose cells that have been differentiated *in vitro* do not perfectly resemble to adipocytes found in adipose tissue (Fig. 1B). From a structural perspective, differentiated 3T3-L1 cells contains mostly multilocular LDs within the cytoplasm compared with the unilocular LDs that are found in *in vivo* adipocytes (Green and Meuth, 1974). Furthermore, in contrast to fat cells of adipose tissue, adipogenic cell lines such as 3T3-L1 do not express leptin at high levels when they are differentiated (MacDougald et al., 1995). Interestingly, the low expression of leptin *in vitro* is corrected when adipogenic cell lines are implanted subcutaneously into mice (Mandrup et al., 1997). These results support the idea that additional factors are required for a complete recapitulation of *in vivo* adipocyte development and function.

During adipose tissue formation, lipid accumulation and adipocyte predetermination *in vivo* appear to be uncoupled from each other. Indeed, contrary to 3T3-L1 cells *in vitro*, murine adipocytes do not contain visible lipids upon birth (Birsoy et al., 2011). These 'ghost adipocytes' express terminal adipocyte markers well in advance of the onset of lipid accumulation, suggesting that these genes are involved in priming the adipocyte precursors to accumulate lipids. Lipid accumulation occurs within the first 2 days of postnatal development, concomitantly with the nursing period. As more lipids accumulate in the adipocytes, the lipid-filled adipocytes subsequently express leptin and other genes associated with lipid accumulation (Birsoy et al., 2011).

Development of vertebrate adipose tissue

Mammalian adipose tissue is composed of numerous cell types, with adipocytes representing ~50–60% of the total number of cells (Suga et al., 2008; Eto et al., 2009). Importantly, the adipocyte population accounts for most (~90%) of the volume of adipose tissue (Eto et al., 2009). Other cell types found in adipose tissue include endothelial cells, pericytes, fibroblasts, leukocytes, neurons and preadipocytes (Eto et al., 2009; Sun et al., 2011). Unlike in culture conditions, adipocytes and non-adipocyte cells are in constant communication with each other *in vivo*, which creates a unique nutritional and hormonal microenvironment. In fact, the adipose tissue microenvironment has been suggested to

promote leukocyte recruitment, which has been implicated in several inflammatory conditions including tumor formation (Anderson et al., 2010; Park and Scherer, 2012).

Adipose tissue has the ability to expand in adulthood to cope with periods of chronic positive energy balance. In adult humans, adipocyte turnover rates vary greatly but are estimated to be ~10% per year (Spalding et al., 2008). A recent study suggests that 1–5% of murine adipocytes are replaced each day (Rigamonti et al., 2011). Furthermore, lipids in the adipocytes are also constantly renewed so that TAGs are replaced six times during the average 10-year lifespan of one fat cell (Arner et al., 2011). Given that adipocytes are terminally differentiated and post-mitotic, progenitor cells must exist to expand adipose tissue. However, we still have limited information about the identity, location and origin of adipocyte progenitor cells. Accordingly, two recent studies independently suggest that perivascular cells of murine adipose tissue that express the surface markers CD34, CD24, SCA1, CD140a and CD140b are putative adipocyte progenitor cells (Rodeheffer et al., 2008; Tang et al., 2008). These cells have a high adipogenic potential *in vitro* and *in vivo* and express the zinc finger protein 423 (ZFP423), a transcription factor involved in preadipocyte determination *in vivo* (Gupta et al., 2010; Gupta et al., 2012). Recently it has been shown that another transcription factor, zinc finger protein 521 (ZFP521), can regulate adipocyte development by inhibiting the expression of ZFP423 (Kang et al., 2012).

In addition to the classical lipid-storing unilocular adipocyte, white adipose depots are also composed of multilocular adipocytes that express uncoupling protein 1 (UCP1) and have higher oxidative and thermogenic capacities upon β-adrenergic stimulation than regular adipocytes (Wu et al., 2013). These cells, known as 'brite' or 'beige' adipocytes, arise from a different progenitor cell than the classical white and brown adipocytes (Wu et al., 2012). More recently, bi-potent adipocyte progenitor cells that express PDGFRα, CD34 and SCA1 were characterized. Such cells have been shown to differentiate into white unilocular adipocytes upon high-fat feeding or brown adipocyte (UCP1-positive cells) upon β-adrenergic activation (Lee et al., 2012). Whether these cells are progenitors of white, beige, brown or other uncharacterized adipocytes remains to be investigated.

Normal adipose tissue development is tightly coupled to the formation of new blood vessels (Crandall et al., 1997; Rupnick et al., 2002; Fukumura et al., 2003). Adequate adipose tissue vascularization and blood flow are crucial to support adipocytes with nutrients and oxygen, and to maximize the efficiency of lipid uptake and/or release in response to variations in the nutritional state. Pharmacological inhibition of angiogenesis is associated with a significant reduction in adiposity in both mice and monkeys (Bråkenhielm et al., 2004; Cao, 2010; Barnhart et al., 2011; Sun et al., 2012). Interestingly, in pathological conditions such as obesity, adipose tissue grows independently of vascularization, leading to a poor oxygen and nutrient supply to adipocytes (Hosogai et al., 2007; Rausch et al., 2008; Yin et al., 2009; Kim et al., 2012). These findings indicate that adipocytes under normal conditions interact with different cells and are exposed to a distinct nutritional milieu than those that comprise the adipose tissue of obese patients. Taking into account that nutrients are important modulators of adipose tissue growth, as further discussed in the next section, elucidation of differences of adipocyte milieu and thus nutrient availability in health and disease are of great importance.

Nutritional cues affecting adipose tissue formation

Adipose tissue has an outstanding capacity to modulate its size and function in response to nutritional cues. Extreme variations in adipose tissue mass are observed between under and overfed animals. Indeed, studies have shown that morbidly obese humans can lose as much as 30–40% of their body weight over the first year after undergoing bariatric surgery, a type of intervention that reduces food intake and in some case nutrient absorption (Shah et al., 2006). Such a massive reduction in adipose tissue mass is also observed when hyperphagia associated with leptin deficiency is corrected by leptin administration in mice and humans (Halaas et al., 1995; Farooqi et al., 1999). The close relationship between adipose tissue accumulation and nutrient availability strongly suggests the existence of regulatory mechanisms that allow the rapid adjustment of adipocyte function and metabolism to the nutritional status.

One important protein that has emerged as a key regulator of adipogenesis and adipocyte maintenance in response to nutrient intake is the serine/threonine protein kinase AKT (also known as PKB), which is well conserved from yeast to mammals (Vivanco and Sawyers, 2002). Loss of AKT in mice causes dwarfism and completely blocks adipogenesis (Peng et al., 2003), whereas its constitutive activation is sufficient to induce the differentiation of 3T3-L1 cells into mature adipocytes (Magun et al., 1996). Moreover, mutation in AKT2, one of the three AKT isoforms, leads to partial lipodystrophy in humans (George et al., 2004). The rise in insulin following a meal induces the activation of phosphoinositide 3-kinase (PI3K) in adipocytes, which drives phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P_3] production and activates AKT (Fig. 2). When active, AKT induces the translocation of the glucose transporter protein 4 (GLUT4) to the cell membrane, thereby promoting the uptake of glucose, an important substrate for lipid synthesis. Akt also activates SREBP1, a key transcription factor controlling lipid metabolism (Yecies et al., 2011). The concomitant rise in glucose uptake and the increased SREBP1 activity play a crucial role in promoting lipid accumulation in response to insulin. In addition to lipogenesis, AKT also directly regulates the adipogenic cascade by exacerbating PPARγ activity. Specifically, AKT phosphorylates GATA2 and FOXO1, which promotes the exclusion of these proteins from the nucleus and induces PPARγ expression and activity (Dowell et al., 2003; Nakae et al., 2003; Menghini et al., 2005; Armoni et al., 2006; Fan et al., 2009).

The mammalian (or mechanistic) target of rapamycin (mTOR) is a conserved kinase that senses and integrates a variety of environmental cues to regulate cell and/or organ growth, and homeostasis (Laplante and Sabatini, 2012). mTOR is a constituent of two well-characterized protein complexes, mTOR complex 1 (mTORC1) and mTORC2. From yeast to mammals, nutrients activate mTOR signaling, which in turn triggers the activation of several anabolic processes that are required for cell growth and proliferation (Laplante and Sabatini, 2009). With the emergence of metazoans, the mTOR pathway became wired to signaling pathways that are activated by growth factors, such as insulin (Laplante and Sabatini, 2012). Importantly, AKT plays a central role in relaying insulin signaling to mTORC1 (Laplante and Sabatini, 2012). Several pieces of evidence indicate that mTORC1 activation by nutrients and/or growth factors is an essential molecular event required for adipose tissue maintenance and growth (Fig. 2). The increase in

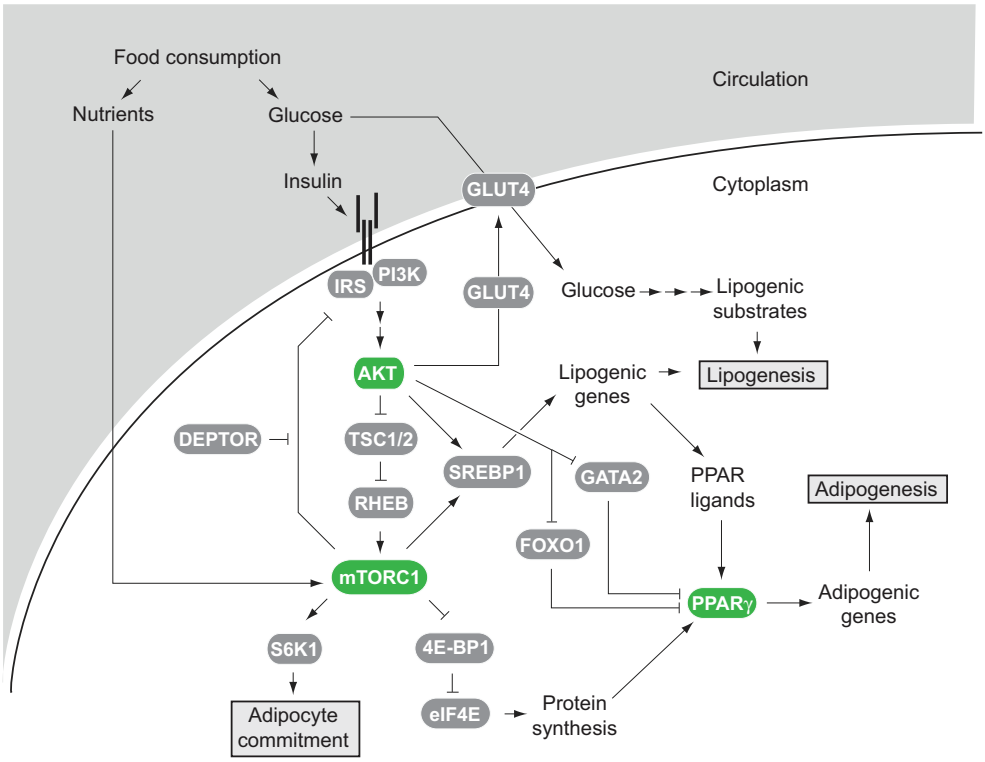

Fig. 2. Regulation of lipogenesis and adipogenesis in mammalian cells by nutrients and insulin. Food consumption leads to a rise in the circulation levels of nutrients and glucose, and promotes the secretion of insulin from the pancreas. The activation of PI3K by insulin promotes AKT and mTORC1 activity, which in turn regulate key complementary steps involved in the promotion of lipogenesis and adipogenesis. Circulating levels of nutrients are sensed by mTORC1 and contribute to the full activation of this protein complex. The coordinated activation of AKT and mTORC1 is essential to drive glucose uptake and metabolism, lipogenic gene expression, PPARγ expression and activation, and adipogenic gene expression, which are all crucial steps in adipocyte formation.

adiposity found upon high-fat feeding, leptin deficiency or pharmacological PPARγ activation is associated with a marked activation of mTORC1 in adipose tissue (Um et al., 2004; Blanchard et al., 2012). Furthermore, pharmacological or genetic inhibition of mTORC1 is associated with a reduction in adipose tissue mass due to both reduced adipocyte size and number (Polak et al., 2008; Houde et al., 2010). mTORC1 appears to affect adiposity by modulating several processes that are involved in adipocyte formation (Fig. 2). mTORC1 regulates the commitment of embryonic stem cells to early adipocyte progenitors by activating S6 kinase 1 (S6K1) (Carnevalli et al., 2010). Furthermore, several studies indicate that mTORC1 activation is also a fundamental step in the terminal differentiation of preadipocytes. mTORC1 overactivation is associated with an increase in adipogenesis *in vitro*, whereas the complete inhibition of mTORC1 by rapamycin or genetic deletion of RAPTOR, a key mTORC1 component, blocks this process (Cho et al., 2004; Kim and Chen, 2004; Polak et al., 2008; Yu et al., 2008; Zhang et al., 2009). mTORC1, through its positive impact on protein translation, promotes the terminal differentiation of adipocytes by regulating the expression of key adipogenic regulators such as PPARγ, CEBPδ and C/EBPα (Le Bacquer et al., 2007). Furthermore, mTORC1 also controls lipid synthesis by directly activating SREBP1 (Bakan and Laplante, 2012).

Taken together, these studies indicate that mTORC1 is a strong positive regulator of fat cell formation. Nevertheless, a recent report from our laboratory suggests that excessive mTORC1 signaling can also have a negative impact on fat cell development (Laplante et al., 2012). We showed that mice overexpressing DEPTOR (DEP domain containing mTOR-interacting protein), a protein that reduces mTOR signaling, unexpectedly accumulate more adipose tissue than control mice. Interestingly, we observed that the *Deptor* locus is part of a quantitative trait locus (QTL) that has been linked to obesity in mice, and that DEPTOR expression is elevated in adipose tissue of obese humans (Laplante et al., 2012). From a mechanistic perspective, DEPTOR promotes adipogenesis by dampening mTORC1 activity, without blocking it. Because mTORC1 overactivation blocks AKT signaling through several negative-feedback loops (Laplante and Sabatini, 2012), we proposed that elevated DEPTOR expression might be required to preserve the pro-adipogenic functions of AKT. These results support a fundamental role of the well-conserved AKT–mTORC1 axis in controlling lipid storage and adipocyte development in response to the nutrients and growth factors.

Concluding remarks

Studying lipid accumulation and/or adipogenesis from lower organisms to mammals has expanded the comprehension of the molecular mechanisms governing adipocyte formation and metabolism. The identification and localization of fat cell precursors along with the discovery of PPARγ and several pro- and anti-adipogenic proteins that regulate adipocyte terminal differentiation represent some of the key discoveries in adipocyte biology. Despite these advances, we should note that many important questions regarding adipocyte development and function still remain unanswered and are likely to be the source of extensive investigation over the years to come. For example, although we now have a better understanding of the identity and the origin of the adipocytes that form adipose tissue, we still have very limited knowledge of the molecular mechanisms that regulate the recruitment of new adipocytes during adipose tissue development and expansion. Do the signals regulating adipocyte commitment come from circulating growth factors and/or nutrients, or emerge directly from enlarged fat cells? How

do these signals turn on PPARγ expression during the early steps of preadipocyte commitment? It is well appreciated that mammalian fat cells express certain factors, such as leptin, to a degree that is proportional to the amount lipid stored in the cells. This suggests that a lipid-sensing mechanism could exist in adipocytes, similar to other nutrient-sensing pathways. The identity of this putative lipid-sensing mechanism in vertebrate adipocytes is still unknown. Identifying such a mechanism is clinically very relevant as this could help the development of new therapeutic avenues to treat obesity and its complications.

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