Reprod. Nutr. Dev. 40 (2000) 325–358

Review article

The adipose conversion process: Regulation by extracellular and intracellular factors

Christophe BOONE[a]*, Jacques MOUROT[a], Francine GRÉGOIRE[b], Claude REMACLE[c]

[a] Unité Mixte de Recherche sur le Veau et le Porc, INRA, 35590 Saint-Gilles, France
[b] Parke-Davis Laboratory for Molecular Genetics, Alameda, CA 94505, USA
[c] Laboratoire de Biologie Cellulaire, Unité de Biologie Animale, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

(Received 19 April 2000; accepted 28 June 2000)

Abstract — White adipose tissue regulates lipid metabolism and acts as a secretory organ. Because of its importance for human health and animal production, many studies have attempted to better understand its development at the cellular and molecular levels by culturing preadipose cells in vitro. This synthesis article describes our current knowledge, acquired by this approach, concerning the regulation of the different steps of the adipocyte differentiation program by extracellular (hormones, cytokines, growth factors, retinoids and fatty acids) and intracellular agents (second messengers and transcription factors). The discrepant effects that have been observed for some of these factors are also discussed. This information is very important in the perspective of a better control of fat deposits in human and breeding species.

preadipocyte / differentiation / hormonal agent / second messenger / transcription factor

Résumé — Le processus d'adipoconversion : sa régulation par des facteurs extracellulaires et intracellulaires. Le tissu adipeux blanc régule le métabolisme lipidique et agit comme un organe de sécrétion. Étant donné son importance pour la santé humaine et la production animale, de nombreuses études ont tenté de mieux comprendre son développement aux niveaux cellulaire et moléculaire en utilisant des cultures de préadipocytes. Cet article de synthèse décrit notre connaissance actuelle, issue de cette approche, concernant la régulation des différentes étapes du programme de la différenciation adipocytaire par des facteurs extracellulaires (hormones, cytokines, facteurs de croissance, rétinoïdes et acides gras) et intracellulaires (seconds messagers et facteurs de transcription). Les effets divergents observés pour certains de ces facteurs sont également discutés. Ces informations sont très importantes dans la perspective d'un meilleur contrôle des dépôts adipeux chez l'humain et les espèces d'élevage.

préadipocyte / différenciation / hormone / second messager / facteur de transcription

* Correspondence and reprints
E-mail: boone@st-gilles.rennes.inra.fr

1. INTRODUCTION

White adipose tissue (WAT) plays an essential role in the regulation of the energy balance of vertebrates. The primary function of the adipocyte is to store energy by accumulating triacylglycerols during excessive energy intake and to restore it during fasting periods. The deposition of fat results from the balance of (1) absorption of blood-circulating fatty acids (FAs) through the action of lipoprotein lipase (LPL), (2) de novo lipogenesis, through plasma glucose and acetate, (3) lipolysis ensured by hormone-sensitive lipase (HSL), and (4) in situ lipid oxidation through β-oxidations in mitochondria. White adipose tissue also acts as a secretory organ. Adipocytes are now recognized to secrete many substances including leptin, the product of *ob* gene known to regulate body fat mass by decreasing food intake and increasing energy expenditure, proteins linked to the immune system (e.g., alternate complement factors (B, C3 and D) and tumor necrosis factor alpha (TNFα)), vasoactive factors (e.g., adenosine, prostaglandins (PGs), angiotensinogen, enzymes required for the conversion of angiotensinogen to angiotensin II, such as renin-angiotensin and non renin-angiotensin systems), proteins of the fibrinolytic and coagulation cascades, such as type 1 plasminogen activator inhibitor (PAI-1) and tissue factor, and adiponectin, also known as apM1 (adipose most abundant gene transcript 1), that can accumulate in vascular walls when the endothelial barrier is injured [4, 7, 48, 93, 122, 131, 175]. Some of these factors can also have a direct action on preadipocyte differentiation. For instance, TNFα and leptin are known for their direct antiadipogenic and/or lipolytic activities, whereas PGI2 and PGE2 are characterized by adipogenic and antilipolytic activities, respectively [4, 16, 52, 93].

People characterized by a dysregulation of their WAT development (e.g., obesity, anorexia nervosa) have increased risk to develop, among others, type II diabetes (NIDDM), hypertension, atherosclerosis and reduced fecundity [153]. On the other hand, fat deposits are also known to influence meat quality [149]. Consequently, a better understanding of the physiological and pathophysiological development of WAT is essential in order to improve treatments against human pathologies as well as for better controlling fat development of commercially important species.

With the establishment of cultures of adipose precursors in vitro, many progresses have been made in our understanding of the adipocyte development at the cellular and molecular levels. The various in vitro models used to study adipocyte differentiation have been reviewed previously [69, 93]. Briefly, two models are mainly used: preadipose cell lines and primary preadipocytes. Preadipose cell lines that originate from mice have been largely investigated. Our current knowledge concerning the general features of the adipose conversion process and its regulation at the extracellular and intracellular levels have been essentially acquired with this culture system, of which the 10T1/2, 3T3-L1, 3T3-F442A, Ob17 and Ob1771 cell lines have been the most studied. On the other hand, primary preadipocytes have been successfully isolated from various animals and cultured in vitro. These cells are far less easy to culture than cell lines, due, for instance, to the process of cell isolation during which several precautions are necessary in order to avoid cell alterations. However, primary cells have the advantage to possess the normal stock of chromosomes and therefore may better reflect the in vivo context. Moreover, it allows comparisons between preadipocytes of different origins since primary cells can be isolated from various animals and from different fat depots, as well as from animals of different physiological states and ages. Two major critics have been addressed to preadipocyte primary cultures. The first is that preadipose cells are at different stages of the adipose conversion process, leading to asynchronous differentiation. The second

is that non-preadipose cells, such as endothelial cells and fibroblasts, have been recovered during the isolation process. However, these contaminant cells are not so numerous than previously suggested since high percentages of lipid-containing cells have already been obtained in primary cultures [23, 68, 92]. Furthermore, we have shown, in a recent study performed with cells isolated from inguinal subcutaneous WAT, that until 80% of the cells can accumulate lipids when cultured in specific conditions (i.e., serum-free medium and supraphysiological concentrations of insulin) [27]. This indicates that no more than 20% of the cells could be non-preadipose cells. A very interesting study, performed by Yu et al. [269] on cultured porcine preadipocytes, strongly suggests that this percentage is probably still smaller. In a first step, these authors depleted, in the presence of complement factors, the preadipocyte population with an antibody developed in their laboratory and recognizing an antigen expressed by preadipose cells, before the expression of the classical early markers of differentiation, such as LPL. Then, they submitted the remaining population to specific conditions (i.e., proliferative medium and glucocorticoids during the proliferation step) and showed that a significant proportion of these cells can be recruited in the adipose pathway. This experiment was very relevant because it demonstrated directly, and for the first time, that the cells that are not able to accumulate lipids in the usual culture conditions (i.e., insulin, glucocorticoids and/or thyroid hormones, generally after the proliferation step or during the whole culture) are probably, at least in part, non-committed cells and/or preadipose cells that are in a very early stage of the adipose conversion process. This study is also a first step for better characterizing the different sub-populations of preadipose cells in porcine primary cultures. A third system of culture has been recently developed. It consists of embryonic stem cells (ZIN 40, E14TG2a and CGR8) derived from the inner cell mass of murine blastocysts, that have been shown to differentiate into fat cells in appropriate conditions. This could provide an excellent model for characterizing *master* genes controlling the commitment of undifferentiated cells into the adipocyte lineage [57].

This review summarizes our current knowledge acquired from in vitro studies that concern the regulation of adipocyte differentiation at extra- and intracellular levels.

2. THE STEPS OF ADIPOSE CONVERSION

Studies with cell lines have demonstrated that numerous steps characterized the adipose conversion process, summarized in Figure 1. These steps were recently reviewed by Grégoire et al. [93]. First, the multipotential stem cells need to be committed to the adipocyte lineage (*determination*) by a process that remains uncharacterized. Interestingly, a recent study showed that mammary stromal cells can differentiate into fibroblasts, adipocytes or capillary-like structures in a hormone- and substratum-dependent manner [271]. The recruited cells, named *adipoblasts*, proliferate (*exponential growth phase*) until reaching confluence, and stop at the G1/S stage of the cell cycle. At that stage, adipoblasts start expressing early markers of adipose conversion, including LPL and α2 chain of type VI-collagen (A2COL6), and are named *preadipocytes*. Expression, secretion and/or organization of numerous extracellular matrix (ECM) components (e.g., type I-, III- and IV-collagens, fibronectin, and laminin), as well as of β-integrins and cytoskeletal proteins/mRNAs (e.g., actin, tubulin, vimentin, vinculin, α-actinin and tropomyosin) also begin to be modified. In cell lines, DNA replication resumes (*clonal expansion*) after the arrest of exponential growth [18] and it was suggested that clonal expansion stops at a precise stage of the G_1 phase of the cell cycle (G_D). In contrast, human primary preadipocytes do not require cell division

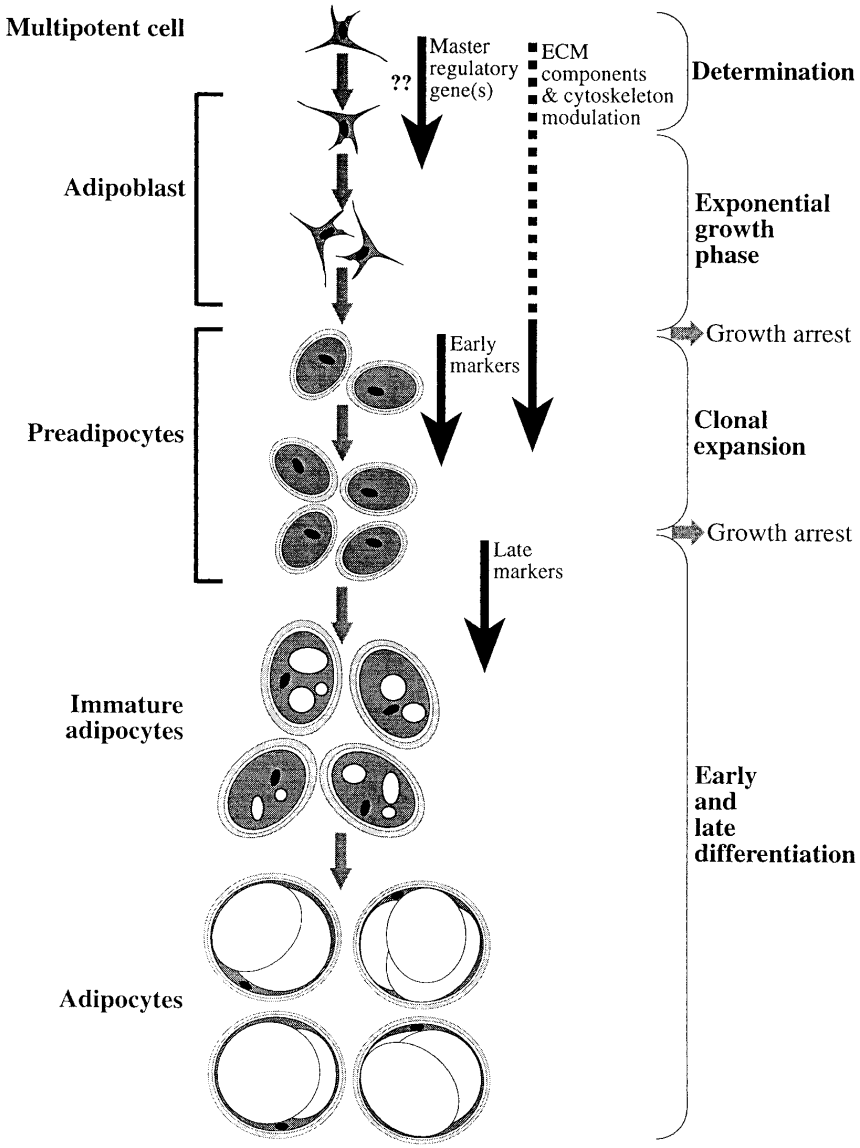

Figure 1. The steps of the adipocyte differentiation program, as observed with the cultures of preadipocytes in vitro. The multipotent cell is recruited in the adipocyte lineage through a process that is still unknown. Indeed, no *master* regulatory gene has been discovered until now. The committed adipose cell, named *adipoblast*, proliferates during the exponential growth phase and the early markers of differentiation, such as lipoprotein lipase, emerge after the withdrawal of this step. This *preadipocyte* performs one or two mitoses (clonal expansion), after what the late markers of differentiation, such as glycerol 3-phosphate dehydrogenase and fatty acid synthase, emerge. The extracellular matrix components and the cytoskeleton are also very implicated in these first steps of the adipose conversion process. The cell starts to accumulate lipid droplets in its cytoplasm (*immature adipocyte*), the nucleus and the cytoplasm being progressively pushed to a peripheral position (*mature adipocyte*).

to enter the differentiation process [68]. After clonal expansion, preadpocytes reach the stage GD' and late markers of differentiation, such as glycerol 3-phosphate dehydrogenase (GPDH), malic enzyme, and FA synthase (FAS), emerge while the morphological differentiation (lipid accumulation) starts [93, 218, 250]. Terminal differentiation of these *immature adipocytes* is marked by a progressive lipid accumulation that will finally push the nucleus to a peripheral position.

3. REGULATION OF ADIPOSE CONVERSION BY EXTRACELLULAR AGENTS

In vitro studies have demonstrated that the various steps of adipocyte differentiation are strongly dependent on hormonal factors, specific FAs and retinoids (Fig. 2).

3.1. Ligands for nuclear receptors

Steroids (e.g., cortisol, vitamin D and sex hormones), triiodothyronine (T_3) and retinoic acid (RA) enter the cells by diffusion. They then interact with their intracellular receptor that belongs to the steroid hormone receptor superfamily (also comprising vitamin D, retinoid and orphan receptors) in a complex fashion still far to be understood. The cytoplasmic steroid receptors form an inactive complex with heat-shock proteins. When the ligand binds the receptor, heat-shock proteins dissociate and homo- or heterodimer receptors can reach the nucleus where they bind to specific steroid response elements. The non-steroid receptors (e.g., receptors for thyroid hormones, RA, and vitamin D) are not associated with heat-shock proteins and can bind DNA as homo- or heterodimers in the absence of their ligand. For instance, binding of RA receptor (RAR) and thyroid hormone receptor to their cognate DNA response elements requires heterodimerization with retinoid X receptor (RXR) whereas orphan receptors can bind as homodimers. On DNA, the unbound receptors are associated to co-repressors and silence active promoters. When ligand binds the receptor, the co-repressor dissociates, allowing interaction with other transcription factors and co-activators, and leading to gene activation [32, 86, 100, 253].

3.1.1. The glucocorticoids

Among steroids, the effects of glucocorticoids on adipose conversion have been the most studied. These hormones are adipogenic in both preadipose cell lines and primary preadipocytes, whatever the conditions of culture [42, 80, 90, 103, 105, 176, 182, 189, 227].

Glucocorticoids have been suggested to act through the PGs but so far results remain conflicting. Gaskins et al. [81] showed that dexamethasone, a synthetic glucocorticoid, decreases the secretion of PGE_2 by porcine preadipocytes whereas Gaillard et al. [80] demonstrated that in Ob1771 cells corticosterone increases secretion of PGI_2 but not of PGE_2. In latter case, PGI_2 would enhance adipose conversion by increasing cyclic AMP (cAMP) and calcium concentrations [245]. In 3T3-L1 preadipocytes, dexamethasone enhanced adipose conversion by inducing the expression of CCAAT/enhancer binding protein (C/EBP)-δ, a protein that belongs to a family of transcription factors ([69, 156]; Sect. 5). The isoform δ is expressed during the exponential growth phase of the adipose conversion [69], suggesting that glucocorticoids play an early role during this process [260, 267]. An early function for dexamethasone, during the growth phase, has also been described in primary porcine preadipocytes. Indeed, the glucocorticoid, in the presence of fetal bovine serum for 3 days after seeding, increases the number of cells expressing the AD-3 antigen, considered by the authors as an early marker of differentiation [259, 269]. The early effect of glucocorticoids is probably due, at least in part, to the induction of

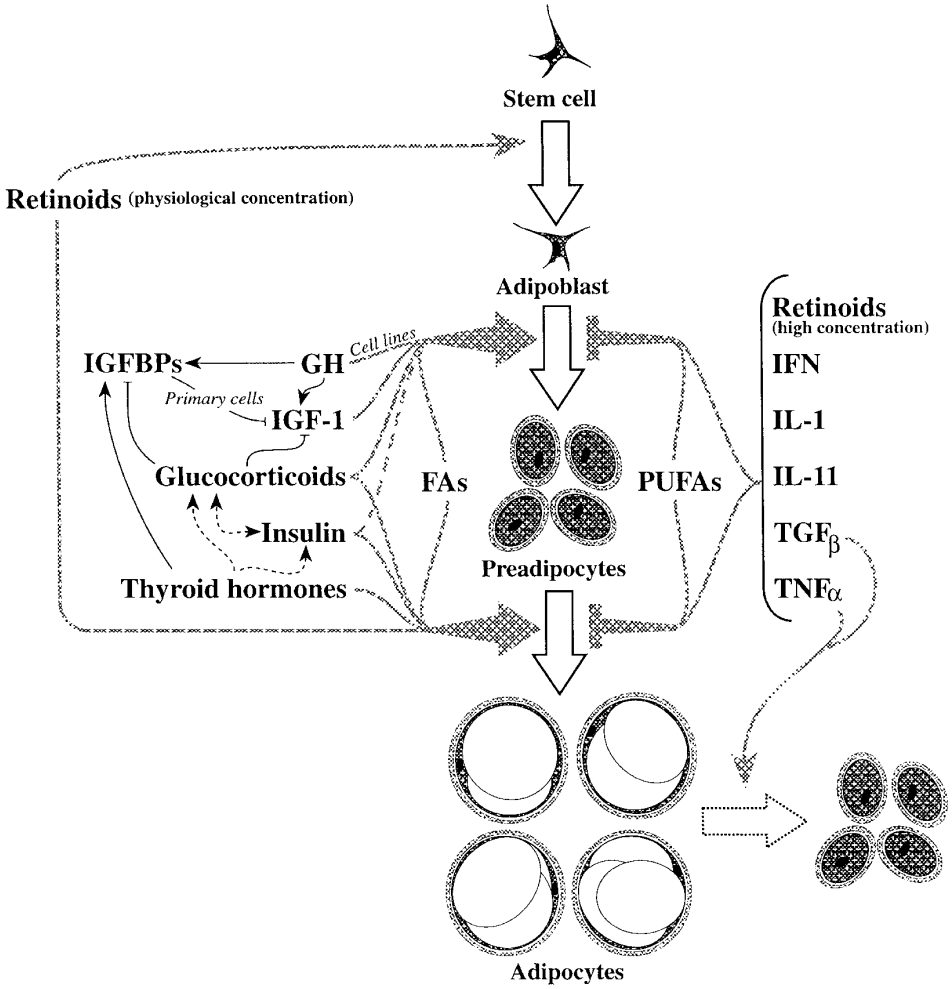

Figure 2. Overview of our current knowledge concerning the regulation of the different steps of the adipose conversion program by extracellular agents. Insulin-like growth factor 1 (IGF-1) stimulates the first steps of the differentiation program. This is also the case for growth hormone (GH) in cell lines whereas a negative effect has been observed with primary preadipocytes. One explanation could be that GH stimulates the transcription of IGF binding proteins (IGFBPs) that can bind and, in some cases, inactivate IGF-1. Glucocorticoids, insulin, retinoids and fatty acids (FAs) enhance both early and late steps of the adipocyte differentiation program whereas T_3 has been shown to have a direct positive impact only during the late steps. Interactions between glucocorticoids, insulin and T_3 have been clearly described. High concentrations of retinoids, polyunsaturated FAs, interferon (IFN), interleukins (ILs) 1 and 11, transforming growth factor β (TGFβ) and tumor necrosis factor α (TNFα) are well characterized as strong inhibitors of the adipose conversion process. A very late inhibitory action (dedifferentiation) has also been observed for TGFβ and TNFα. The case of other factors for which discrepancies have been observed is discussed in the text.

the synthesis of ECM components. Indeed, dexamethasone was reported to enhance the production of laminin and type IV collagen in porcine primary preadipocytes [113, 115]. These ECM components are critical for the development of the adipocytes that are embedded in a basement membrane. In 3T3-L1 cells, dexamethasone has also been shown to stimulate the synthesis of chondroitin sulfate proteoglycans, known to increase during the adipose conversion process [33, 213]. Smas et al. [214] as well as Wolf [257] recently demonstrated that glucocorticoids can also promote adipogenesis by repressing Pref-1, a transmembrane protein that is largely expressed in 3T3-L1 preadipose cells but is absent in mature adipocytes [212]. An impact of glucocorticoids during the late steps of adipose conversion is not to be excluded since the number and affinity of their receptors increases during this process [44, 99, 110].

3.1.2. The thyroid hormones

High-affinity binding sites for T_3 have been described in Ob17 preadipocytes, their number doubling during adipose conversion [83]. Stimulation of these cells by T_3 alone increased, albeit in a limited extent, the activity of adipose conversion-related enzymes (e.g., FAS and GPDH), and an additive adipogenic effect was obtained with insulin. A recent study showed that T_3 receptors $\alpha 1$ and $\beta 1$, but not $\beta 2$, are strongly implicated in the adipogenic action of T_3 in the same cell line [55]. The $\beta 1$ type might have a particularly important role during the early steps of adipose conversion, since it is expressed only for 2 days from the growth arrest, whereas the $\alpha 1$ type is expressed during a longer period. This hormone increases adipose conversion (i.e., number of adipocytes as well as GPDH and malic enzyme activities) of 3T3-F442A cells, but only in presence of fetal bovine serum, indicating that T_3 is not adipogenic by itself [73]. No direct study has been performed on 3T3-L1 cells but it has been observed that these preadipocytes do not need T_3 to differentiate [103, 195]. Furthermore, T_3 seems to have no impact on primary preadipocytes isolated from rabbit [182]. This hormone is classically added to culture human preadipose cells [59, 68, 106] but its requirement has never been clearly demonstrated. Interestingly, a recent study has demonstrated that this hormone can have an adipogenic impact, albeit limited, during late differentiation of porcine preadipocytes [27]. Experiments with rat preadipose cells suggested that the effect of T_3 may depend on the presence or not of serum. Indeed, no effect was observed on GPDH activity in serum-containing medium [255] whereas, in chemically-defined medium, its removal clearly decreased LPL and GPDH activities [60]. These studies pointed out the complexity of the action of T_3 and indicate that this hormone would essentially act indirectly on adipose conversion, by modulating other factors. In vivo experiments performed on fetal pigs showed that thyroxine increases lipogenesis, an effect that is counterregulated by growth hormone (GH), and suggested that this thyroid hormone enhances tissue development by increasing serum and tissue concentrations of insulin-like growth factor 1 (IGF-1) as well as of IGF binding proteins (IGFBPs) [116, 148].

The presence of T_3 response element (T_3RE) in malic enzyme gene promoter indicates that T_3 receptors can act directly on gene promoters of adipose conversion-related enzymes [61, 180, 268]. Gonzalez-Manchon et al. [84] have suggested that, in the absence of T_3, binding of T_3 receptors homodimers to T_3RE represses human malic enzyme gene expression. These homodimers would dissociate from the T_3RE when T_3 is present and the receptors could then heterodimerize with RXRα, this new complex activating malic enzyme gene expression. A recent study indicates that the expression of this gene can also be repressed by type β-T_3 receptors through a DNA-independent mechanism [85]. This hormone has also been shown to regulate stearoyl-CoA

desaturase gene and acetyl-CoA carboxylase (ACC) PI promoter [123, 251]. However, further investigations should be performed to better understand the role played by T_3. This is necessary since T_3 was shown to stimulate malic enzyme mRNA synthesis in rat liver, heart and kidney but not in other rat tissues including brain, lung and spleen [171].

Interestingly, thyrotropin (TSH), that stimulates T_3 and thyroxine production, has recently been shown to directly enhance proliferation of rat preadipocytes, whereas it inhibits their adipose conversion [102].

3.1.3. The retinoids

Vitamin A can be metabolized into at least three active retinoids that are named all-*trans* retinoic acid (*t*-RA), 3,4-didehydroretinoic acid (ddRA) and 9-*cis* retinoic acid (9-*cis* RA). Two families of nuclear RA receptors have been described: the RAR, binding *t*-RA and 9-*cis* RA, and the RXR, binding 9-*cis* RA, each one including three receptor isoforms (i.e., α, β and γ). These receptors act through the formation of RAR-RXR heterodimers or RXR-RXR homodimers. In addition, RXR can heterodimerize with other nuclear receptors, such as the T_3 receptor and peroxisome proliferator-activated receptor (PPAR) [21, 87, 141, 150, 273].

The RARα, RARγ, RXRα and RXRβ mRNAs are abundant in adipose tissue, and have been detected in 3T3-L1 and Ob1771 cell lines, especially in committed preadipocytes and differentiated cells [101, 130, 190]. In Ob1771 cell line, physiological concentrations of *t*-RA or 9-*cis* RA increase the GPDH activity [190]. The authors also observed stimulatory effects using physiological concentrations of synthetic retinoids, and demonstrated that only a very small proportion of RAR molecules need to be activated to obtain the maximal adipogenic effect. In addition, they showed that RARα should particularly be implicated in this process because small concentrations of RARα agonist were sufficient to increase GPDH activity. It is noteworthy that treatment with the RARα agonist Am580 did not influence the expression of early markers of differentiation (i.e., A2COL6 and LPL), suggesting that adipogenic effects of RAs are mostly effective during the terminal differentiation process. However, a critical role for RA has also been demonstrated during the early steps of the adipocyte differentiation, since pretreatment of differentiating embryonic stem cell-derived embryoid bodies with *t*-RA for a short time results in a high degree of adipogenesis [57]. Finally, in primary cultures, physiological concentrations of *t*-RA increase adipose conversion of rat preadipocytes but do not influence differentiation of porcine preadipose cells [190, 226].

By contrast, supraphysiological concentrations of *t*-RA and/or 9-*cis* RA prevent adipose conversion of Ob1771 and 3T3-L1 cells, and cause death of 3T3-L1 cells by apoptosis [43, 145, 190, 192]. In this cell line, high doses of RA completely block the differentiation-related reduction in *rrg*/lysyl oxidase gene expression, an enzyme that is able to alter ECM composition [63]. High concentrations of RA also inhibit adipose conversion of 3T3-F442A cells probably by altering, at least in part, the assembly of actin microfilaments [39]. The adipose conversion of porcine and bovine preadipocytes has also been shown to be inhibited by supraphysiological concentrations of *t*-RA and/or vitamin A [174, 226]. Interestingly, Ohyama et al. [174] mentioned that they previously showed that vitamin A inhibits adipose conversion of ovine preadipose cells at concentrations corresponding to its plasma level in sheep. The inhibitory effect of RA appears to be early because it can block adipogenesis by inhibiting the transcription mediated by C/EBPβ [196], an isoform that is expressed during the exponential growth phase of the adipose conversion process (Sect. 5). The observation that RA needs to be added for only 24 h at early stage of

culture in porcine cell cultures argues also for an early action of these molecules [226]. However, wathever the origin of the preadipocytes, pharmacological doses of RA inhibit both early (e.g., A2COL6 and LPL) and late (e.g., GPDH and adipsin) markers of differentiation [5, 13, 224, 234]. Tontonoz et al. [239] hypothesized that stimulation of RAR by RA could block terminal differentiation by leading to RAR-RXR heterodimerization preventing the formation of PPARγ2-RXRα heterodimers. This would be consistent with the observation that RAR suppresses the action of PPARγ and is supported by the fact that the actions of RA are mostly mediated by RAR [132, 262]. Xue et al. [262] also showed that RAR can inhibit PPARγ mRNA expression, correlated with a decrease in the level of PPARγ protein. These results strongly suggest that the inhibitory effect of RA is ensured, at least in part, through impairment of PPARγ-stimulated transcriptions. Therefore, RA can have a dual action on adipose conversion (during early and late steps), depending on its concentration. The molecular mechanisms remain to be clarified.

3.1.4. The fatty acids

In addition to their role in the production of energy and in the formation of phospholipids and PGs, long-chain FAs also act as transcriptional regulators, whereas short-, middle- and very long-chain FAs remain poor inducers [6, 19, 94, 243, 244]. Several FA binding proteins (FABP) have been described [244]. Some of them are associated to the plasma membrane, such as the membrane FABP, the FA transport protein (FATP) and the FA translocase (FAT). These proteins ensure the entry of plasma FAs, mainly bound to albumin, into the cell by a mechanism that is still discussed. Others are cytoplasmic and could facilitate the transport of the FAs to their site of utilization. Two different intracellular FABPs have been described in adipose tissue, the adipocyte lipid-binding protein (A-LBP), also known as aP2, and the less abundant keratinocyte lipid-binding protein (K-LBP) [19, 244].

The FATP and FAT have been shown to be expressed during adipocyte differentiation of 3T3-F442A and Ob1771 cells, and to be closely linked to this process [1, 2, 194, 206]. Similarly, activation of the cytoplasmic A-LBP gene, that is currently used as a differentiation marker, appears at the beginning of the triacylglycerol accumulation, just following the emergence of other early markers (e.g., LPL, A2COL6) [6].

In addition to the esterification process that lead to triglyceride storage in the adipocyte, FAs are also implicated in the regulation of the adipose conversion process [94]. For instance, exposure of Ob1771 cells to palmitate promotes post-confluent mitoses, accumulation of triglycerides and emergence of late markers of adipose differentiation (e.g., GPDH activity) [10]. Amri et al. [9, 12] also showed that long-chain FAs activate the expression of A-LBP, LPL and acyl-CoA synthetase, an enzyme that is induced during adipose conversion and is important for starting lipid deposition. On the other hand, polyunsaturated long-chain FAs (PUFAs) can inhibit adipose conversion, at least in part through decrease in PPARγ and C/EBPα expressions. PUFA-specific response element, acting as repressor of transcription, has been described in the promoter of stearoyl-CoA desaturase gene [30, 252]. Furthermore, Ntambi et al. [173] demonstrated that PUFAs decrease stearoyl-CoA desaturase gene expression in hepatocytes, and Fukuda et al. [77] showed that, in hepatocytes and adipocytes, PUFAs can suppress the insulin stimulation of FAS transcription. It is noteworthy that activation of adipose conversion has also been observed with PUFAs [193].

Of interest is that long-chain FAs can enhance adipose conversion by binding directly and activating PPARγ, that is able to regulate the expression of A-LBP and FATP [6, 76, 239, 256]. Recent studies also

indicated that A-LBP functions as a positive factor in FA signaling by directly targeting and delivering FA metabolites to the lipid signal transduction pathway [118].

Like most adipogenic inducers, FAs act synergistically with other agents such as GH [10] and retinoids for controlling the adipose conversion process and lipid metabolism [134, 191]. In addition, insulin can phosphorylate A-LBP, reducing its affinity for FAs. Consequently, this increase in unbound FAs could up-regulate expression of lipid-related genes [6].

3.2. Extracellular agents acting through transmembrane receptors

Protein hormones, growth factors and cytokines act through transmembrane receptors that activate various intracellular pathways. Insulin, IGF-1, epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) bind to transmembrane receptors containing tyrosine kinase domains whereas GH and cytokine receptors do not possess tyrosine kinase activity but mediate their effect through cytoplasmic kinase proteins. On the other hand, transforming growth factor (TGF)-β stimulates a receptor containing serine/threonine kinase activity, whereas catecholamines, prostaglandins, adenosine and angiotensin-II bind G protein-associated receptors [35, 52, 58, 100, 157, 160, 164, 197, 217].

3.2.1. Insulin, IGF-1, and GH

Insulin belongs to the most adipogenic factors but its impact depends on the cellular model. Supraphysiological concentrations of insulin enhance adipose conversion of preadipose cell lines by acting through the IGF-1 receptor [215]. Cross-reactions with this receptor is indeed possible when insulin is added at high concentrations [45, 140]. Because no or poor effects are observed with physiological concentrations, similar interpretation concerns human and rabbit preadipocytes [105, 182]. However, it has been shown more recently that high concentrations of insulin are not obligatory for the differentiation of 3T3-L1 cells [223]. Moreover, physiological concentrations are able to increase adipose conversion of rat and porcine cells, even if supraphysiological concentrations remain more efficient [27, 60, 90, 109]. These results suggest that, at least in these cases, insulin is also able to have adipogenic effects by acting directly on its own receptor.

Insulin and glucocorticoids are able to modulate the action of each other. For instance, insulin increases the number and affinity of glucocorticoid receptors in porcine preadipocytes from fetal origin [44, 110]. Synergistic effects between these two hormones were also described in primary preadipocytes from different origins [90, 105, 182, 227]. Furthermore, it was suggested that insulin could enhance the adipose conversion of porcine preadipocytes that have been previously recruited by glucocorticoids [113, 269]. It is noteworthy that such interactions also depend on the origin of the cells, since rodent preadipocytes are able to sustain the adipose conversion process with insulin alone, in the absence of glucocorticoids [60, 90]. In contrast, glucocorticoids are more adipogenic than insulin in TA1 cell line and primary rabbit preadipose cells [42, 182]. In 3T3-F442A cells, insulin is only effective when cells have been previously stimulated with GH [95]. However, an earlier role of insulin is not to be excluded: this hormone is indeed sufficient to enhance adipose conversion of porcine preadipocytes in the absence of other stimulators, and to increase the expression of extracellular matrix proteins, such as laminin and type IV collagen [27, 113].

Growth hormone has also been reported to enhance adipose conversion of preadipose cell lines by acting through IGF-1. Experiments on 3T3-F442A cells suggested that GH would enhance adipose conversion

by triggering the entrance of precursor cells into the differentiation program, by exiting the cell cycle and establishing quiescent primed cells. These cells would be in a special G0 stage of the cell cycle that is permissive for differentiation [50, 89, 97]. Then, as shown in 3T3-F442A and Ob1771 cells, GH would stimulate the transcription of the IGF-1 gene, as also demonstrated for porcine preadipocytes [82], and sensitize the cells to the mitogenic effect of IGF-1 by stimulating the expression of its receptor. This would lead to clonal expansion that would selectively increase the number of these newly recruited cells [65, 89, 272]. The surprising negative effect of glucocorticoids on 3T3-F442A cell line has been explained by the fact that GH binding is down-regulated by these hormones, probably through a decrease in the number of GH receptors [138]. Other studies performed on 3T3-F442A cells showed that GH also up-regulates cytoskeleton proteins, such as vinculin and tubulin, leading to the changes in cell shape that are necessary for adipose conversion [26, 96, 98, 221].

Studies on 3T3-L1 cells did not corroborate these results since exogenous IGF-1 was obligatory and sufficient for the differentiation of these cells [215]. In addition, data collected on primary cultures showed that IGF-1 enhanced adipose conversion by increasing the preadipocyte pool, whereas GH had no effect or even a negative effect [27, 112, 114, 249]. Some of the differences observed between cell lines and primary cells could be explained by the fact that primary cells are most likely harvested in a later stage of adipose conversion and may have been stimulated in vivo by hormonal agents, such as GH [68]. On the other hand, the inhibitory effect of GH in primary preadipocytes appears contradictory since GH enhances the secretion of IGF-1 in primary culture. It has been hypothesized that this negative effect might be mediated, at least in part, by stimulating the secretion of IGFBPs that can block the adipogenic action of IGF-1 [46, 186]. These authors also showed that IGF-1 and IGFBP secretions are controlled by thyroxine, glucocorticoids and TGFβ. Finally, the action of IGF-1 is strongly dependent on the age of the animal since preadipocytes from porcine fetus produce more IGF-1 and respond better to this hormone than cells from neonate pigs [45]. Furthermore, the various isoforms of IGFBPs are divergently produce by fetal (preferentially IGFBPs 2 and 3) and postnatal (preferentially IGFBPs 1 and 4) preadipocytes [45].

3.2.2. Other growth factors and cytokines

Although in vivo experiments have shown that EGF and TGFα, that also acts through the EGF receptor, are associated to an inhibition of adipose conversion [154, 199, 201, 204], the results obtained in vitro remain controversial. Studies on 3T3-L1 cells revealed that EGF increases adipose conversion in serum-free medium [15] whereas negative effect were obtained in serum-containing medium [3]. Furthermore, EGF increases the differentiation of porcine preadipocytes [27], whereas it inhibits the adipose conversion of rodent and human preadipose cells cultured in similar conditions [107, 198, 247]. Transforming growth factor α, that can be expressed by preadipose cells, exhibits antiadipogenic activities when tested on 3T3-F442A and rat preadipose cells [154, 198, 199]. Studies on primary cells suggest that EGF exerts its function during the early stages of the adipose conversion program. In rat preadipocyte cultures, the expression of EGF receptors is increased early during the differentiation process, then slightly decreased during the late steps. Such receptors are even undetectable in mature adipocytes directly isolated from rats [78]. Similar observations have been made with human preadipocytes [75]. An early impact of EGF on adipose conversion is also supported by the observation that, in porcine preadipocyte cultures, this growth factor strongly increases LPL

activity, an early marker of differentiation, whereas its impact on malic enzyme activity, a late marker, is not so conclusive [27].

The effects of PDGF and fibroblast growth factor (FGF) are also still controversial since positive and negative effects have been reported for cell lines and primary preadipocytes. These effects depend on the growth factor concentration, the medium composition and/or the origin of the cells. Treatment with PDGF increases adipose conversion of 3T3-L1 cells [15, 223] whereas it decreases differentiation of TA1 cells [166]. No effect is observed on the adipose conversion of human preadipocytes [107]. Studies on 3T3-L1 cells showed that the omission of PDGF lead to loss of differentiation as well as to cell death by apoptosis [223]. Platelet-derived growth factor can also act by modulating other growth factors. For example, in the 3T3 cell system, PDGF modulates EGF receptor by inducing a transient loss of EGF binding activity, independently of any change in EGF receptor affinity [258]. In serum-containing medium, FGF was shown to decrease the differentiation of TA1 cells [166, 167]. In contrast, FGF had no or a poor effect on the adipose conversion of 3T3-L1 cells or of human and rat primary preadipocytes cultured in serum-free medium [107, 195, 200, 247]. Recently, FGF-10 was shown to be produced by rat preadipocytes and to ensure their growth without affecting differentiation [263].

Transforming growth factor β is able to block early and/or late steps of adipose conversion in TA1, Ob1771, 1246 and 3T3-T cell lines [56, 128, 219, 240]. Similar results were reported for rat, porcine and human primary preadipocytes [179, 185, 186, 247]. As suggested for PDGF and FGF, this negative effect appears to be independent on a proliferative action [185, 240]. Investigations on 3T3-T cells suggested that the action of TGFβ could be mediated by a protein kinase C (PKC)-dependent pathway by blocking an event of the cell cycle that occurs after the arrest in the GD stage, but prior to the GD' stage, of the cell cycle [220]. One study also suggested that TGFβ should act, at least in part, by increasing the synthesis of fibronectin and collagen [125]. Studies on porcine cells showed that it inhibits secretion of adipogenic agents such as IGF-1, and increases IGFBPs secretion [185, 186]. This hormonal agent could act in an autocrine/paracrine manner since it is locally produced by porcine adipocytes in vivo and in vitro [184]. Consistently with its inhibitory effect, its synthesis is decreased during the adipose conversion of 3T3-L1 cells [254]. Finally, TGFβ can also reduce the expression of adipose genes in mature adipocytes [240].

Tumor necrosis factor α, interferon γ, and interleukins 1 and 11 strongly decrease adipose conversion in cell lines as well as in rat, porcine and human primary cells [22, 27, 56, 91, 133, 178, 240, 247]. The mechanism of action of TNFα has been largely investigated. It can act directly on lipid metabolism by decreasing FA uptake (e.g., decrease in LPL, FAT and FATP expression) and lipogenesis (e.g., decrease in FAS and ACC expression), and by increasing lipolysis [205]. Indirect effects have also been observed since this cytokine can alter collagen (types I, III, and IV) and β-actin synthesis in 3T3-L1 cells [254]. These authors also showed that TNFα could exert its effect by increasing the TGFβ mRNA content. Another interesting observation is that TNFα disrupts clonal expansion by changing the normal pattern of expression of p107 and p130 proteins, known to play a role during this step that is necessary to the adipose conversion process (Sect. 5) [155, 187]. The cells then enter the S phase but undergo apoptosis. As observed with TGFβ, TNFα can reduce the expression of adipose genes in mature adipocytes. However, in both cases, these "dedifferentiated" cells are distinguishable from preadipocytes since one cannot detect some factors that are expressed in preadipocytes, such as Pref-1 [74, 240, 261, 274].

3.2.3. The prostaglandins

Preadipocytes and adipocytes produce large amount of PGs, including PGI_2, $PGF_{2\alpha}$ and PGE_2 [124]. Prostaglandin $F_{2\alpha}$ inhibits adipose conversion of cell lines and rat preadipocytes [124, 169, 202, 203]. Because this PG can stimulate the synthesis of TGFα in rat preadipocytes, the existence of an amplification mechanism between these two differentiation inhibitors has been postulated [199]. By contrast, carbaprostacyclin ($cPGI_2$), a stable analogue of PGI_2, is recognized as a strong auto-/paracrine adipogenic agent for Ob1771 cells as well as for primary rodent and human preadipocytes [40, 170, 245, 247]. In addition to PGI_2, PGD_2 and its derivative PG 15-deoxy-J_2 may be endogenous ligands for PPARγ [117, 142]. The roles of PGD_2 and PGE_2 are however still controversed since divergent effects have been observed according to the cell culture model [37, 203]. Despite these discrepancies, interesting models are emerging concerning the auto-/paracrine effects of PGE_2 and PGI_2 [52, 53, 58, 246]. As mentioned above, fat cells can release angiotensinogen that is a precursor of the vasoconstrictor angiotensin II, which has been shown recently to play an important role in the cell cycle progression of human preadipocytes [53]. In addition, by binding to its receptor in adipocytes, angiotensin II stimulates the production of PGI_2 and PGE_2. The PGI_2 receptor is essentially present in preadipose cells and PGI_2 would then increase cAMP concentration in undifferentiated cells, enhancing their adipose conversion. By contrast, PGE_2 receptors are mostly present in mature adipocytes. Consequently, PGE_2 would decrease cAMP concentration, avoiding lipolysis. In conclusion, these PGs lead to an increase of adipose tissue mass by enhancing hyperplasia and hypertrophy. Interestingly, adenosine, by acting on its A1 and A2 receptors, can lead to effects similar to those obtained with PGE_2 and $cPGI_2$ respectively [28]. Transfection of Ob17 cells with the cDNA of human A1 adenosine receptor even initiated the adipose conversion [232]. The authors then suggested that these receptors could act through cell-to-cell contacts at confluence. 15-deoxy-delta PGJ_2 does not need to activate membrane receptor since it is a natural ligand for PPARγ [211].

3.3. Conclusion

The complex hormonal regulation of the adipose conversion has been studied extensively in vitro. Several discrepancies essentially due to the various origins of the preadipose cells and to the different culture conditions have emerged from these studies. However, they have highlighted a general scheme that includes the moment of the hormonal effect during this process (Fig. 2).

The hormonal agents that have been shown to play the most important adipogenic functions during the adipocyte differentiation are glucocorticoids, insulin, IGF-1, T_3 and GH. Several works performed on cell lines and primary cultures clearly indicate that glucocorticoids play an important adipogenic function during the early steps of adipose conversion. This early impact of glucocorticoids would prepare the cells to respond better to the adipogenic impact of insulin during the late steps of this process. This could explain the synergistic impact of glucocorticoids and insulin that has been observed on late adipose conversion. Indeed, if some studies suggested that glucocorticoids could also have adipogenic functions during late adipocyte differentiation, this has not been clearly demonstrated. Similarly, the possible early impact of insulin remains to be proved. IGF-1 is recognized, in both preadipose cell lines and primary preadipocytes, to play an early role during the adipose conversion process, by selectively increasing the number of preadipose cells. However, as shown for porcine preadipocytes, its positive impact on adipose conversion could be affected by the local production of IGF-BPs, under the control of glucocorticoids, thyroid hormones and GH. The case of GH appears more

dependent on the origin of the cells but, in both systems of culture, this hormone has been shown to enhance the transcription of the IGF-1 gene. Further studies will be necessary to understand exactly why GH exerts adipogenic functions in cell lines whereas anti-adipogenic impacts have been described in primary cells, as discussed in Section 7. On the other hand, some studies performed with cell lines and primary cultures showed that T_3 can directly increase late adipose conversion, albeit in a limited extent. However, this hormone seems to exert its effect by preferentially modulating the activities of other adipogenic hormonal agents, such as insulin and glucocorticoids. All together, these in vitro investigations have allowed to dissect the mechanisms of action of hormones during the different steps of adipose conversion. Moreover, they have highlighted the interactions existing between these various hormones. Additional work is needed to extend these observations but this first scheme is a very interesting complement for the in vivo context that should help to better understand WAT development.

Cytokines have been shown to interfere with the adipocyte differentiation. Indeed, TGFβ, TNFα, IL-1, IL-11 and IFN exhibit strong antiadipogenic effects, through various modes of action, in both preadipose cell lines and primary preadipocyte systems. A dedifferentiation of mature adipocytes has even been observed with TGFβ and TNFα. Cytokines are well known for their role in the coordination of the immune response and the link between immunity and WAT is reinforced by the fact that this tissue is able to produce various proteins of the immune system, among others TGFβ, TNFα and complement factors [93]. In addition, human obesity is accompanied by high plasma concentrations of TNFα that are responsible of insulin resistance. This reinforces the interest to better understand and characterize the mechanisms of action of these agents, that are a link between immunity, WAT development and some physiological dysregulations, in the context of economically important animals.

The actions of other hormonal factors are not so clear. This is the case of EGF, TGFα, PDGF and FGF for which adipogenic and antiadipogenic effects have been observed according to the study. This is also the case for PGD_2 and PGE_2. On the other hand, PGI_2 is well known for its adipogenic effects whereas $PGF_{2\alpha}$ is a strong inhibitor of adipose conversion.

During this last decade, several works have described the role of long chain FAs during the adipose conversion process. Positive (e.g., by increasing the number of preadipocytes or by increasing the activity of early and late markers of differentiation, such as LPL and GPDH respectively) and negative effects (e.g., through a decrease in transcription factors that play an important role during adipose conversion, such as PPARγ and C/EBPα) have been described according to the concerned FAs. Such dual impact is particularly important since FAs can be added in the diet for controlling adipose tissue development of economically important species (e.g., by addition of specific oils). Physiological concentrations of retinoids, that are synthesized from vitamin A, are able to increase adipose conversion in cell lines and primary cultures, mainly through RAR activation. The observation that retinoic acid can recruit stem cells in the adipocyte lineage make these lipophilic molecules very interesting, mostly in the perspective of an early control of the adipose tissue development in meat-producing animals. As observed for FAs, retinoids are characterized by a dual effect since they can inhibit adipose conversion when added at supraphysiological concentrations. Consequently, these molecules could also be used for better controlling fat development in vivo. However, further studies are necessary to determine the exact effects of retinoids, for example on the general physiology of the animals, especially in the case of introduction of supraphysiological concentrations.

4. REGULATION OF ADIPOSE CONVERSION BY SECOND MESSENGERS

The hormonal agents acting through transmembrane receptors activate numerous intracellular pathways that are progressively better understood [35, 100, 157, 160, 164, 197]. Specific regulation of these downstream events could explain some of the divergent adipogenic and/or antiadipogenic effects observed with hormonal factors according to the origin of the preadipocytes. The complexity of the regulation of adipose conversion is increased by the fact that the intracellular pathways can be activated and/or modulated by ECM components, through integrins [49, 151]. On the other hand, the discovery of cAMP-response element binding protein (CREB) binding proteins (CBPs) pointed out the high level of interactions between nuclear hormone receptors and the intracellular pathways induced by the hormones binding membrane receptors [127].

In the precise case of the adipocyte differentiation, the downstream events implicated in the adipogenic action of insulin are the best characterized until now, as clearly reviewed by Sorisky [217]. The small G-protein Ras, phosphoinositide 3-kinase (PI3-kinase), protein kinase B (PKB) and mitogen-activated protein kinases (MAPK) have been shown to be implicated. Janus kinase (JAK)-2 and signal transducer and activator of transcription (STAT)-5 appear to be important intracellular pathways used by GH for inducing adipose conversion of 3T3-F442A cells, whereas neither MAPK nor S6 kinase are necessary [266]. In addition, c-Fos and PKC appear to be implicated in the action of GH on the adipose conversion of Ob1771 cells [66].

Among the intracellular events, cAMP and PKC pathways belong to the most common pathways activated by transmembrane receptors and several works demonstrated that they strongly affect the adipocyte differentiation.

4.1. The cAMP pathway

Numerous studies, in which cAMP pathway has been stimulated by either plasma membrane permeant cAMP analogs (i.e., 8-bromo-cAMP and dibutyryl-cAMP), forskolin or methylisobutylxanthine (MIX, a nonselective inhibitor of phosphodiesterases), suggested that this pathway plays a pivotal role in the adipocyte differentiation process. Treatment of 3T3-L1 cells with forskolin, permeant cAMP analogs or MIX clearly increases GPDH activity and/or the expression of stearoyl-CoA desaturase mRNA [29, 36, 103, 195]. Similar observations were performed with 3T3-F442A [265] and Ob1771 cells [40, 79, 245]. This adipogenic activity of cAMP pathway was further supported by the observation that adenosine A2 receptor and $cPGI_2$, that act through this pathway, increase the GPDH activity in Ob1771 preadipose cells, as well as in rat and human primary preadipocytes [28, 40, 245, 247].

Cyclic AMP pathway appears to play a role early during adipose conversion, including the clonal expansion step. Indeed, in 3T3-L1 cells, cAMP simulates the A-LBP promoter in confluent preadipocytes but not in proliferating or fully differentiated adipocytes [264]. The authors also concluded that cAMP does no act through cAMP response element but relieve the inhibitory effect of a negative regulatory element blocking the expression of this gene. In addition, cAMP-elevating agents increase stearoyl-CoA desaturase mRNA expression in preadipocytes but not in adipocytes [36]. The authors suggested that this cAMP-regulated stearoyl-CoA desaturase expression could play a role that differs from the lipogenic functions ensured by this enzyme during late differentiation. One interesting hypothesis is that stearoyl-CoA desaturase, by producing unsaturated FAs, could alter membrane fluidity and then protein binding, conformation and function. As mentioned above, FAs and derivatives can act as signaling molecules during the adipose

conversion process, for instance through PPARs, and stearoyl-CoA desaturase could provide such molecules. In Ob1771 cells, the cAMP pathway was suggested to induce a transition from the G_0 to the G_1 phase of the cell cycle, allowing clonal expansion and then the commitment of a maximal number of cells able to respond to the adipogenic hormonal stimuli [79].

By contrast, some studies showed that the activation of the cAMP pathway is not always associated to an increase in adipose conversion. Indeed, dibutyryl-cAMP in combination with theophylline (a phosphodiesterase inhibitor) was shown to prevent lipid accumulation in 3T3-F442A cells [222]. Recently, it has been demonstrated that these cells respond divergently to cAMP-elevating agents according to their concentrations and the moment of the treatment [265]. In addition, forskolin, isoproterenol and MIX are able to potentiate GH-dependent differentiation when added just after the confluence, whereas they inhibit the adipogenic effect of insulin when added during terminal differentiation. Finally, studies on porcine preadipocytes were shown to be unable to respond to cAMP-elevating agents whatever the culture medium, the concentrations and the moment of utilization [24]. Further investigations are needed to explain such diverging effects but a differential intracellular regulation according to the species is not to be excluded.

4.2. The protein kinase C pathway

By contrast with the cAMP pathway, PKC is generally recognized as a strong inhibitor of adipose conversion. Protein kinase C activity is decreased during the adipose conversion of 3T3-L1 cells and the addition of phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), that, at nanomolar concentrations, directly activates the PKC activity by mimicking diacylglycerol [172], decreases the proportion of differentiated cells [207, 241]. These results are supported by the observation that staurosporin, an inhibitor of PKC, increases both the proportion of differentiated adipose cells and GPDH activity [241]. Similar observations were performed with the 3T3-T, TA1 and A31T cell lines [62, 166, 167, 220, 270], as well as with rat primary cultures [208, 209].

Protein kinase C appears to exert its antiadipogenic activity at an early stage of differentiation since TPA was shown to inhibit adipose conversion of 3T3-L1 cells only when added concomitantly with the inducers of adipose conversion, for 48 h from confluence [166]. One hypothesis is that TPA blocks the entry of the cell into the G_D stage whereas studies on 3T3-T cells suggested that PKC pathway could block the adipose conversion program after this stage [220]. The addition of various inhibitors of PKC to TA1 cells, for 24 h from 1 day before confluence, led to a drastic decrease in the expression of clone 5 mRNA, a differentiation marker of these cells [166].

However, several PKC activators enhance terminal differentiation of Ob1771 cells [40] and TPA exert both mitogenic and adipogenic activities on porcine primary preadipocytes [23]. In addition, negative and/or positive modulation of adipose conversion by PKC have been reported to be mediated through a replicative/proliferative effect during the clonal expansion phase, but TPA has also been shown to inhibit differentiation independently of cell proliferation [40, 62, 166, 207, 220, 270]. These results indicate that this pathway of signal transduction belongs to a complex system able to have positive and negative effects on the adipose conversion process. The origin of such divergences remains to be investigated. These divergent effects can be explained by the fact that PKC pathway is regulated in a complex manner and that numerous PKC isoforms exist [161, 236]. These isoforms may have distinct biological

functions [31, 54, 121, 147, 165, 242]. Moreover, they are differentially expressed and have different effects (i.e. adipogenic, anti-adipogenic or no effects) during the adipose conversion of 3T3-F442A preadipocytes [71]. In addition, PKCζ, a non-DAG/TPA-activated subunit, could be involved in the mechanisms underlying the variations in the proliferating and differentiating capacities of preadipocytes according to their anatomical localization [147, 159, 242]. Therefore, according to the origin of the cells, it is possible that the activation of specific PKC subtypes may increase or decrease adipose conversion. This is supported by the fact that PKCβ can be expressed and have an adipogenic role in 3T3-L1 but not in 3T3-F442A preadipocytes [71, 159, 242].

4.3. Conclusion

Studies on second messengers clearly highlight the complexity of the regulation of adipocyte differentiation by second messengers, as well as possible species specificities. Indeed, whereas cAMP is generally recognized as a strong stimulator of adipose conversion in rodent species, no effect has been observed on porcine preadipocytes, whatever the stimulator, its concentration and the moment of stimulation. Similarly, whereas PKC generally strongly inhibits differentiation of rodent preadipocytes, excepted in the case of Ob1771 cells, a very significant increase of this process has been obtained with porcine preadipose cells. The fact that PKC can enhance adipose conversion of Ob1771 cells, as observed for porcine preadipocytes, suggests that some of these divergences could be due to other reasons than species specificities (e.g., preadipose cells at different stages of adipose conversion or different culture media).

Further studies are necessary to determine the exact origins of these discrepancies that possibly explain, at least in part, the diverging impacts observed with some hormonal agents according to the origin of the cells (Sect. 3). Such information is of high interest for our understanding of the regulation of WAT development in vivo since they could point out different regulational mechanisms according to the species, the depots or even the physiological status of the animal.

5. THE FUNCTIONS OF TRANSCRIPTION FACTORS AND OTHER INTRACELLULAR AGENTS DURING THE STEPS OF THE ADIPOSE CONVERSION PROCESS

During this last decade, transcription factors have been investigated extensively, improving our knowledge concerning their functions in the adipose conversion process (Fig. 3).

The molecules of the PPAR and C/EBP families are transcription factors containing a leucine zipper domain for dimerization or heterodimerization (e.g., with RXR and C/EBP homologous protein 10 (CHOP-10), respectively) and a basic domain for their binding on DNA. Several isoforms have been described (i.e., PPARα, β (= δ) and γ; C/EBPα, β and δ) and cooperate for enhancing adipose conversion during the various steps of adipose conversion. PPARα and β are able to increase adipose conversion but the most adipogenic is PPARγ [51, 69, 156].

5.1. The arrest of exponential growth

PPARγ and C/EBPα expression is especially increased during clonal expansion but the low levels detected before could be sufficient for enhancing cell cycle withdrawal during the exponential growth phase [69, 156]. In addition, in some cell lines such as PAZ6 preadipocytes, PPARγ has been shown to be well expressed at confluence [225]. In the human fibrosarcoma cells HT1, Timchenko et al. [235] demonstrated that

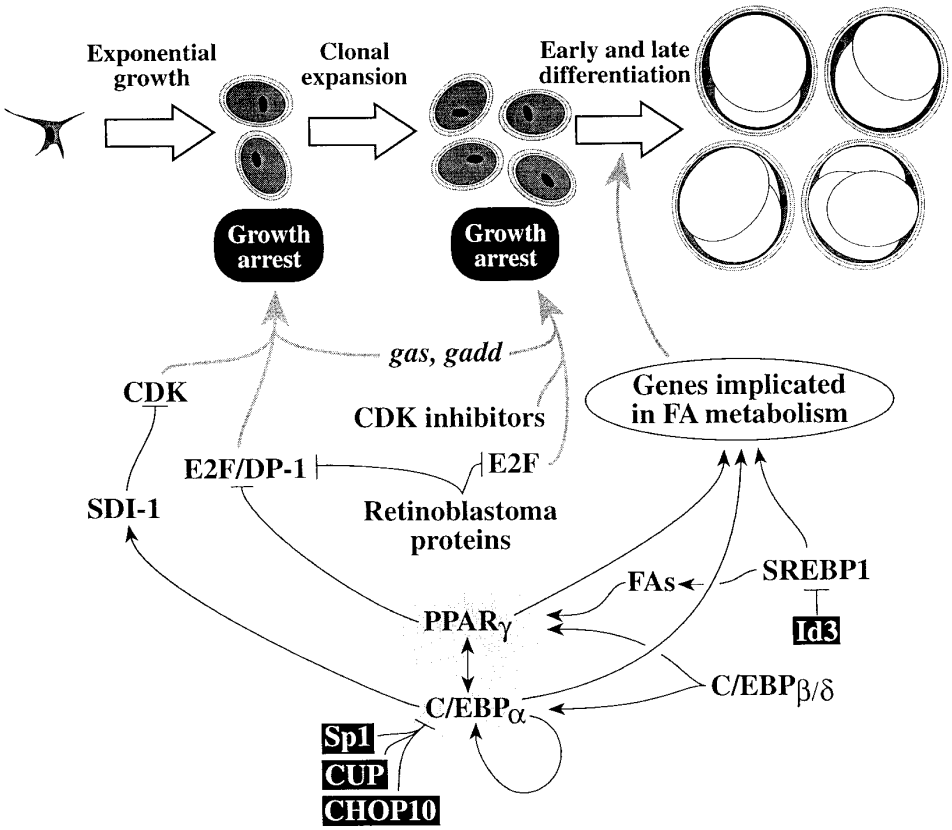

Figure 3. Overview of our current knowledge concerning the regulation of the different steps of the adipose conversion program by intracellular agents. The control of the two growth arrests is very determinant for the early steps of the adipocyte differentiation process. The most important mechanisms implicated in this regulation are the inhibition of E2F (a transcription factor that activates proteins implicated in the cell cycle) by PPARγ and retinoblastoma proteins, the actions of the growth arrest-specific (*gas*) and of the growth arrest- and DNA damage-inducible (*gadd*) genes, and the inhibition of the cyclin dependent kinases (CDKs), for instance by SDI-1 that is under the control of C/EBPα. The late steps of the adipose conversion program are under the control of PPARγ, C/EBPs and SREBP1. The specific inhibitors of C/EBPα and SREBP1 are in black boxes.

induction of C/EBPα caused inhibition of cell proliferation and DNA synthesis by increasing gene expression of p21/SDI-1, as well as by stabilizing this protein, that is an inhibitor of the cyclin-dependent kinase (CDK). On the other hand, Altiok et al. [8] showed that activation of PPARγ in the adipogenic HIB1B cells leads to a growth arrest as well as to a decrease in DNA binding and transcriptional activities of the E2F/DP-1 complex. This effect was accompanied by an increase in the phosphorylation of these proteins as well as by a decrease in the expression of the catalytic subunit of the serine-threonine phosphatase PP2A. The authors then hypothesized that PPARγ could stop exponential growth phase by inhibiting the transcription of genes that are associated with entry into S phase and DNA synthesis since E2F-binding sequence has been

described in the promoters of such genes [146]. C/EBPβ and γ, that are preferentially expressed during the early steps of adipose conversion and then disappear while clonal expansion arrest occurs, have the function to increase the expression of PPARγ and C/EBPα, as discussed later, but a role during this early step is not to be excluded [69, 156].

5.2. The arrest of clonal expansion

Regulation of cell growth withdrawal after clonal expansion has also been investigated. The CDK inhibitors p18, p21 and p27 are probably implicated in this growth arrest since this step corresponds to an increase of their mRNA/protein expression, that is under the positive control of PPARγ in the case of p18 and p21 [163, 181]. A recent study demonstrated that the calcium-activated protease calpain degrades p27, allowing the preadipocytes to re-enter the cell cycle after confluence and to undergo clonal expansion and terminal differentiation [177]. As observed during the exponential growth phase, the cell growth stimulator complex E2F/DP is inactivated during growth arrest of clonal expansion. In 3T3-L1 cells, such a role could be attributed to two proteins of the retinoblastoma tumor suppressor gene family, p107 and p130, that can bind E2F [187]. Indeed, at confluence, E2F predominantly binds to p130 and very few free E2F is detectable. When clonal expansion starts, p130 protein level decreases whereas p107 protein increases and free E2F appears, available for transcription. The initial pattern is recovered when clonal expansion stops. Another member of the retinoblastoma tumor suppressor gene family, the retinoblastoma protein (pRb), is also able to block E2F activity and has been linked to adipogenesis [51]. In addition, it has been shown that pRb becomes phosphorylated during clonal expansion and that it increases the binding affinity of C/EBPα and β on C/EBP response elements. Growth arrest-specific (*gas*) and growth arrest- and DNA damage-inducible (*gadd*) genes probably also play important roles in withdrawal, from growth phase and/or clonal expansion, since they are differentially expressed at distinct growth arrest points, at confluence or at the end of post-confluent mitoses [210].

5.3. The early and late adipocyte differentiation

In cell lines, C/EBPα and PPARγ expression strongly increases during clonal expansion and the positive effect on adipose conversion suggests that they prepare the cells for terminal differentiation by activating several adipogenic genes [69, 156]. In addition, adipocyte determination and differentiation factor 1/sterol regulatory element-binding protein 1 (ADD1/SREBP1), a member of the bHLH (basic region/helix-loop-helix domain protein) family, is known to induce genes implicated in the FA metabolism (e.g., types 1 and 2 stearoyl-CoA desaturase genes) by binding on specific DNA binding sites, such as the E-box, a helix-loop-helix consensus sequence, and the sterol response element [51, 69, 228, 237]. A model of action for C/EBPs, PPARγ and ADD1/SREBP1 has been proposed [69, 156]. C/EBPβ and C/EBPδ are expressed early and activate PPARγ and C/EBPα expression. On the other hand, ADD1/SREBP1 also enhances PPARγ activity probably by initiating the production of endogen FAs issued from lipid metabolism. Indeed, this transcription factor is able to induce FAS and LPL, two key enzymes of FA metabolism, and it has been shown that cells expressing ADD1/SREBP1 produce and secrete lipid molecule(s) that bind directly to PPARγ [135, 136]. Because PPARγ can also induce C/EBPα expression (and vice versa), it is possible that the role of ADD1/SREBP1 would be to maintain PPARγ, and then C/EBPα expression, when C/EBPβ and C/EBPδ concentrations are lower [69, 156]. C/EBPα is also able to autoactivate itself by binding on the

promoter of its own gene [156]. Interestingly, Castillo et al. [38] recently cloned a novel protein, termed PGC-2, that is able to bind PPARγ, but not the other PPARs, contributing to its adipogenic action. The case of PPARδ is not so well documented but this isoform is expressed very early and could have a function before the expression of PPARγ [11].

5.4. The inhibitors of the adipogenic transcription factors

On the other hand, the action of these transcription factors can be downregulated by other transcription factors. Indeed, CHOP10 is able to heterodimerize with C/EBPα and then to avoid its binding on CCAAT binding sites. Similarly, inhibitor of DNA binding-3 (Id3) can heterodimerize with ADD1/SREBP1, avoiding its binding on E-box [69]. However, the expression of Id4 is increased during 3T3-L1 differentiation, suggesting that it plays a role during this process [47]. In the same cell line, Tang et al. [230] showed that Sp1, a retinoblastoma control protein, binds the C/EBPα promoter early during the adipose conversion program, prior to the stimulation of differentiation and then to post-confluent mitoses. This prevents the binding of C/EBPs and then its transactivation. Stimulation of these cells by cAMP-elevating agents decreases Sp1 level early in the differentiation program. This allows the access of C/EBPβ and/or C/EBPδ to the promoter of C/EBPα. Similarly, another transcription factor, C/EBPα undifferentiated protein (CUP), also known as AP-2α, binds and repress C/EBPα transcription [129, 229]. As observed for Id3, the expression of CUP is decreased during the adipose conversion process [69, 129].

5.5. Conclusion

In vitro studies have allowed to better understand the functions of various transcription factors during the adipose conversion process (Fig. 3).

Three transcription factors are particularly important: PPARγ, C/EBPα and ADD1/SREBP1. PPARγ and C/EBPα appear to act on both exponential growth arrest, by interfering with factors implicated in the cell cycle regulation, and terminal differentiation, through activation of adipogenic genes. On the other hand, ADD1/SREBP1 is essentially involved during this last event, by activating genes implicated in FA metabolism, as well as by enhancing PPARγ activity.

C/EBPβ and δ play an indirect, but essential, role on terminal adipose conversion by activating PPARγ and C/EBPα expression.

The regulation of clonal expansion growth arrest appears independent of PPARγ, C/EBPs and ADD1/SREBP1. As described for exponential growth arrest, a control of factors involved in the cell cycle regulation, essentially exerted by retinoblastoma proteins in this case, is clearly implicated. Despite the absence of observation until now, we cannot exclude such a control by retinoblastoma proteins during exponential growth arrest.

It is noteworthy that repressor factors have been discovered, especially for the activity of C/EBPα and ADD1/SREBP1.

As assessed by these results, our knowledge concerning the regulation of adipose conversion by transcription factors is significantly increasing. There is still very few data that have emerged from primary cultures despite of their interest, as demonstrated by the variations that have been observed concerning the regulation of adipose conversion by hormonal agents and second messengers. In addition, it has been shown recently that, when compared to cell lines, porcine adipose precursor cells express C/EBPα and PPARγ very early [111, 137]. The authors then suggested that porcine preadipocytes are probably more advanced in development than 3T3-L1 cells. These considerations clearly demonstrate that

investigating the expression and function of transcription factors in primary preadipocytes is an inevitable step in the perspective to better understand and interpret, at long term, the regulation of adipose conversion in an in vivo context.

6. NEW INSIGHTS IN CONTROLLING FAT DEVELOPMENT

Despite the discovery of numerous intracellular factors implicated in adipose conversion, the list is far to be exhaustive. Regularly, new agents playing a role during this process are identified. Their functions are not always well defined and it will be very important to carefully study these new agents in order to increase our knowledge concerning the regulation of the adipocyte differentiation.

Uncoupling proteins (UCPs) are well known for uncoupling the respiratory chain reactions from ATP synthesis in mitochondria [119, 188]. Three UCP homologues have been described: UCP1 is restricted to brown adipose tissue, UCP2 has been found in several tissues, including WAT, and UCP3 is expressed preferentially in skeletal muscle and brown adipose tissue [72, 248]. Aubert et al. [14] showed that UCP2 mRNA expression increases during adipose conversion of Ob1771 and 3T3-F442A cells. The fact that the gene of PIL-7, coding for a subunit of cytochrome C oxidase, was similarly expressed indicates that some mitochondriogenesis is taking place during adipose conversion, probably playing a role in the energy expenditure of mature adipocytes. The authors also suggested that PPARδ could play an important role in the regulation of UCP2 mRNA expression in preadipocytes. The implication of PPARs in UCP2 mRNA expression was also suggested by Camirand et al. [34]. However, in the human PAZ6 cells, in which this mRNA expression is also increased during the adipose conversion, it was demonstrated that the effects of PPARs activators reflect a general increase in adipocyte differentiation rather than a specific increase of UCP2 mRNA expression [225].

The transcription and activity of semicarbazide-sensitive amine oxidase (SSAO), an enzyme known for its positive impact on glucose transport, are largely increased during the adipose conversion of 3T3-L1 and 3T3-F442A cells [162]. The authors showed that both expression and activity of SSAO can be modulated by pathways implicated in the adipose conversion process, such as cAMP pathway and TNFα, suggesting that this enzyme could be involved in the regulation of adipocyte homeostasis.

The *murine double minute-2* (*mdm-2*) gene, coding for a protein that is able to block the MyoD-mediated myogenesis [70], is more expressed in 3T3-L1 preadipocytes than in A31 cells that lack the possibility to differentiate [17]. However, this expression remains elevated in adipocytes and the results suggest that adipogenesis is unaffected by elevated Mdm-2 protein level. By contrast with other cell types in which Mdm-2 interacts with proliferation stimulators, such as p53 [108, 144], the overexpression of *mdm-2* gene appears independent on p53, and Mdm-2 could mediate proliferation-independent effects.

Ho et al. [120] recently demonstrated that nuclear factor of activated T cells (NFAT) is present in both preadipocytes and adipocytes but has a DNA binding activity only in mature adipocytes. They also showed that NFAT increases A-LBP gene expression whereas the inhibition of its nuclear localization impairs adipose conversion. The authors suggested that NFAT could act after PPARγ induction but before C/EBPα induction, raising the possibility that the induction of the C/EBPs genes could be themselves controlled by members of NFAT family.

Another very interesting way of investigations concerns the redox signaling. Indeed, superoxide and hydrogen peroxide, two

inorganic molecules known for their destructive actions on organic structures, are also used as signaling pathways within cells and can be produced in response to hormonal factors such as PDGF, EGF, IL-1 and TNFα [100, 183]. In the case of adipose cells, May and de Haen [158] observed that hydrogen peroxide can mimic the stimulatory effects of insulin on glucose transport and lipid synthesis. However, the mechanisms of action of these molecules, in the context of their cellular functions, remain unclear. Albeit poorly studied until now, this signaling pathway is more and more investigated and the results should have very important impacts in our understanding of the regulation of differentiation programs such as adipose conversion.

Finally, many studies of adipose conversion concerns the identification of probable *master* gene(s), responsible of the commitment of pluripotential cells into the adipogenic lineage, such as MyoD in the myogenic lineage [231]. Several candidates have been proposed for the adipogenic program. For instance, C/EBPα and PPARγ cooperate for inducing the commitment of NIH-3T3 fibroblasts into the adipocyte lineage [238]. However, although these transcription factors are expressed early during the adipose conversion process, no detection before confluence, a time at which the cells have already started their adipogenic program, has been performed until now [69, 225]. An interesting system of culture, that can be used for better characterizing probable *master* gene(s), has been developed by Dani et al. [57]. As mentioned above, it consists of cultures of embryonic stem cells derived from the inner cell mass of murine blastocysts. The authors showed that treatment of these cells with retinoic acid leads to adipocyte differentiation, indicating that the receptors and pathways involved are probably intimately linked to probable *master* gene(s). Recently, in 3T3-L1 cells, Imagawa et al. [126] identified 58 clones corresponding to genes that are induced very quickly after the addition of the adipogenic factors. A better identification of these genes could highlight new regulators and/or new interactions during the early steps of adipose conversion.

7. DIVERGENCES ACCORDING TO THE ORIGIN OF THE CELLS

Studies performed on preadipose cell lines highlighted the general features allowing an undifferentiated cell to accumulate lipids and develop into adipocyte. In addition, many studies have attempted to define the hormonal and intracellular regulation of this process [5, 69, 93]. However, some divergences have been observed according to the origin of the cells. This can be explained by the fact that cell lines are at different stages of the adipose conversion program. For instance, 10T1/2 cells express multipotential characteristics since they can differentiate, among others, into preadipocytes, premyocytes or prechondrocytes [233]. Other cells can only differentiate into adipocytes, such as TA1, 30A5, 3T3-L1, 3T3-F442A and Ob17 [41, 88, 143, 168]. Such differences are not surprising because these cells are obtained from different protocols. For example, 3T3-L1 and 3T3-F442A cells have been cloned from Swiss 3T3 cells, originated from Swiss 3T3 mice embryo [88] whereas Ob17 preadipocytes have been obtained from epididymal WAT of adult ob/ob obese mice [168]. In addition, we cannot exclude that the aneuploid caryotype of the preadipocyte cell lines might explain some of the divergences observed between cell lines and/or between cell lines and primary preadipose cells.

One of the most striking example of divergences concerns the 3T3-F442A cells. These cells appear strongly dependent on GH for ensuring their adipose conversion [50, 97] whereas 3T3-L1 and Ob1771 cells are less dependent on this hormone [64, 80, 103, 215]. This difference might be responsible for the negative effect of glucocorticoids observed on 3T3-F442A cell

differentiation, since glucocorticoids are though to decrease the number of GH receptors in this cell line [138], whereas these lipophilic hormones are highly adipogenic in adipocytes of other origins [42, 80, 103]. Consequently, the effects of GH in 3T3-F442A cells, as well as their intracellular consequences [96, 98], remain difficult to extrapolate to preadipocytes of other origins. However, this indicates that 3T3-F442A cells might be in a different stage of adipose conversion and the information accumulated with this cell line could be of interest for describing the regulation of preadipocyte regulation in this particular step of the adipose conversion process.

Cultures of primary preadipocytes, directly isolated from the animals, were developed because they reflect better the in vivo context. Their interest was demonstrated by the fact that their adipose conversion process exhibits some differences when compared to that of cell lines. These studies were essentially performed with rat preadipose cells but preadipocytes from murine, rabbit, ovine, porcine, bovine and human species were also successfully cultured [27, 67, 92, 107, 113, 152, 174, 182, 216]. These cells can be isolated from different fat depots, as well as from animals of different ages and/or different physiological state [92, 104, 139]. These experiments showed that the regulation of adipose conversion is strongly dependent on the origin of the cells, and pointed out species specificities. For instance, the primary preadipocytes are generally considered to be in later steps of adipose conversion than cell lines [5, 68]. In addition, preadipose cells isolated from fetus or young animals differentiate better than those obtained from older animals [20, 45]. Grégoire et al. [92] also showed that obese-derived preadipocytes differentiate poorly, when compared to lean-derived cells, probably due to differences in their stage of commitment. Finally, studies performed in our laboratory showed that porcine preadipose cells do not respond like preadipocytes of other origins to EGF, cAMP-elevating agents and PKC stimulators [23, 24, 27]. These differences observed between the regulation of porcine adipocyte differentiation and that of other species has been previously reviewed [25].

8. CONCLUSIONS

These last decades, many studies have attempted to better understand the development of the adipocyte differentiation at cellular and molecular levels. Despite some variations according to the study, essentially due to the preadipocyte origin and/or to the culture conditions, some general features emerged concerning the regulation of the different steps of this complex process.

Many efforts have been made to study the dependence of adipocyte development by hormonal agents. Glucocorticoids and IGF-1 appear to be the most efficient hormones during the early adipose conversion whereas insulin is the most adipogenic hormonal agent during the late steps. Some studies on cell lines suggest that IGF-1 also plays an important function at this time. The actions of T_3, GH and EGF seem to be more dependent on the origin of the preadipocytes. The roles of other growth factors and cytokines are not to be neglected since they can influence adipose conversion through numerous pathways, such as the cell cycle, the extracellular matrix, the cytoskeleton, the synthesis of other hormonal agents and apoptosis. Retinoids and FAs also regulate adipocyte differentiation. If the role and mechanism of action of retinoids remains to be defined, they could belong to the first events implicated in the commitment of multipotential cells into the adipogenic pathway. On the other hand, recent studies clearly demonstrated the central role assumed by FAs since they can control, among others, their own metabolism.

Several studies have attempted to describe the intracellular pathways that are necessary for adipocyte differentiation.

These investigations showed that PPARγ, C/EBPα, β, δ and ADD1/SREBP1 intimately cooperate to ensure adipose conversion, the activities of C/EBPs and ADD1/SREBP1 being inhibited by CHOP-10 and Ids, respectively. Retinoblastoma proteins (pRb, p107, p130), E2F and p21/SDI-1, *gas* and *gadd* enhance or stimulate the adipose conversion process by interfering, at least in part, with the cell cycle. Interactions have been demonstrated between some of these factors. The roles of other agents, classically classified as second messengers because they directly transfer signals transmitted by transmembrane receptors, have also been investigated. This is the case of cAMP and PKC that appear to enhance of inhibit the adipose conversion during the early steps of this process.

These numerous studies that were performed with both preadipose cell lines and primary preadipocytes have helped to better understand the regulation of the adipose conversion program at the cellular and molecular levels. However, more efforts are needed to extend this knowledge. This will allow to better treat obesity and obesity-related disorders, as well as to better control fat development in breeding species.

REFERENCES

[1] Abumrad N.A., Forest C.C., Regen D.M., Sanders S., Increase in membrane uptake of long-chain fatty acids early during preadipocyte differentiation, Proc. Natl. Acad. Sci. USA 88 (1991) 6008–6012.

[2] Abumrad N.A., El-Maghrabi M.R., Amri E.-Z., Lopez E., Grimaldi P.A., Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation, J. Biol. Chem. 268 (1993) 17665–17668.

[3] Adachi H., Kurachi H., Homma H., Adachi K., Imai T., Morishige K.-I., Matsuzawa Y., Miyake A., Epidermal growth factor promotes adipogenesis of 3T3-L1 cell in vitro, Endocrinology 135 (1994) 1824–1830.

[4] Ailhaud G., L'adipocyte, cellule sécrétrice et endocrine, Médecine/Sciences 14 (1998) 858–864.

[5] Ailhaud G., Grimaldi P., Négrel R., Cellular and molecular aspects of adipose tissue development, Annu. Rev. Nutr. 12 (1992) 207–233.

[6] Ailhaud G., Abumrad N., Amri E.-Z., Grimaldi P., A new look at fatty acid as signal-transducing factors, in: Galli C., Simopoulos A.P., Tremoli E. (Eds.), Fatty acids and lipids: biological aspects, Vol. 75, World Rev. Nutr. Diet-Basel, Karger, 1994, pp. 35–45.

[7] Alessi M.C., Peiretti F., Morange P., Henry M., Nalbone G., Juhan-Vague I., Production of plasminogen activator inhibitor I by human adipose tissue: possible link between visceral fat accumulation and vascular disease, Diabetes 46 (1997) 860–867.

[8] Altiok S., Xu M., Spiegelman B.M., PPARγ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A, Genes Dev. 11 (1997) 1987–1998.

[9] Amri E.-Z., Ailhaud G., Grimaldi P., Regulation of adipose cell differentiation. II. Kinetics of induction of the aP2 gene by fatty acids and modulation by dexamethasone, J. Lipid. Res. 32 (1991) 1457–1463.

[10] Amri E.-Z., Ailhaud G., Grimaldi P., Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells, J. Lipid. Res. 35 (1994) 930–937.

[11] Amri E.-Z., Bonino F., Ailhaud G., Abumrad N.A., Grimaldi P., Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes, J. Biol. Chem. 270 (1995) 2367–2371.

[12] Amri E.-Z., Teboul L., Vannier C., Grimaldi P., Ailhaud G., Fatty acids regulate the expression of lipoprotein lipase and activity in preadipose and adipose cells, Biochem. J. 314 (1996) 541–546.

[13] Antras J., Lasnier F., Pairault J., Adipsin gene expression in 3T3-F442A adipocytes is post-transcriptionally down-regulated by retinoic acid, J. Biol. Chem. 266 (1991) 1157–1161.

[14] Aubert J., Champigny O., Saint-Marc P., Négrel R., Collins S., Ricquier D., Ailhaud G., Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells, Biochem. Biophys. Res. Commun. 238 (1997) 606–611.

[15] Bachmeier M., Löffler G., Influence of growth factors on growth and differentiation of 3T3-L1 preadipocytes in serum-free conditions, Eur. J. Cell Biol. 68 (1995) 323–329.

[16] Bai Y., Zhang S., Kim K.S., Lee J.K., Kim K.H., Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipogenic hormones, J. Biol. Chem. 271 (1996) 13939–13942.

[17] Berberich S.J., Litteral V., Mayo L.D., Tabesh D., Morris D., mdm-2 gene amplification in 3T3-L1 preadipocytes, Differentiation 64 (1999) 205–212.

[18] Bernlohr D.A., Bolanowski M.A., Kelly T.J., Lane M.D., Evidence for an increase in transcription of specific mRNAs during differentiation of 3T3-L1 preadipocytes, J. Biol. Chem. 260 (1985) 5563–5567.

[19] Bernlohr D.A., Ribarik Coe N., LiCata V.J., Fatty acid trafficking in the adipocyte, Cell Dev. Biol. 10 (1999) 43–49.

[20] Björntorp P., Karlsson M., Pettersson P., Sypniewska G., Differentiation and function of rat adipocyte precursor cells in primary culture, J. Lipid Res. 21 (1980) 714–723.

[21] Blanchet S., Michaille J.-J., Les RXR ne sont pas des partenaires d'hétérodimérisation purement passifs, Médecine/Sciences 14 (1998) 1211–1216.

[22] Boney C.M., Fiedorek F.T., Paul S.R., Gruppuso P.A., Regulation of preadipocyte factor-1 gene expression during 3T3-L1 cell differentiation, Endocrinology 137 (1996) 2923–2928.

[23] Boone C., Grégoire F., De Clercq L., Remacle C., 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulates the adipose conversion of piglet preadipocytes in primary culture, Cell. Mol. Biol. 44 (1998) 1265–1274.

[24] Boone C., Grégoire F., Remacle C., Various stimulators of the cyclic AMP pathway fail to promote adipose conversion of porcine preadipocytes in primary culture, Differentiation 64 (1999) 255–262.

[25] Boone C., Grégoire F., Remacle C., Regulation of porcine adipogenesis in vitro, as compared with other species, Domest. Anim. Endocrinol. 17 (1999) 257–267.

[26] Boone C., Grégoire F., De Clercq L., Remacle C., The modulation of cell shape influences porcine preadipocyte differentiation, In Vitro Cell. Dev. Biol. 35 (1999) 61–63.

[27] Boone C., Grégoire F., Remacle C., Culture of porcine stromal-vascular cells in serum-free medium: differential action of various hormonal agents on adipose conversion, J. Anim. Sci. 78 (2000) 885–895.

[28] Borglum J.D., Vassaux G., Richelsen B., Gaillard D., Darimont C., Ailhaud G., Négrel R., Changes in adenosine A1- and A2-receptor expression during adipose cell differentiation, Mol. Cell. Endocrinol. 117 (1996) 17–25.

[29] Brandes R., Arad R., Benvenisty N., Weil S., Bar-Tana J., The induction of adipose conversion by bezafibrate in 3T3-L1 cells. Synergism with dibutyryl-cAMP, Biochim. Biophys. Acta 1054 (1990) 219–224.

[30] Brodie A.E., Manning V.A., Ferguson K.R., Jewell D.E., Hu C.Y., Conjugated linoleic acid inhibits differentiation of pre- and post-confluent 3T3-L1 preadipocytes but inhibits cell proliferation only in preconfluent cells, J. Nutr. 129 (1999) 602–606.

[31] Cacace A.M., Guadagno N., Krauss R.S., Fabbro D., Weinstein I.B., The epsilon isoform of protein kinase C is an oncogene when overexpressed in rat fibroblasts, Oncogene 8 (1993) 2095–2104.

[32] Calkhoven C.F., Ab G., Multiple steps in the regulation of transcription-factor level and activity, Biochem. J. 317 (1996) 329–342.

[33] Calvo J.C., Rodbard D., Katki A., Chernick S., Yanagishita M., Differentiation of 3T3-L1 preadipocytes with 3-isobutyl-1-methylxanthine and dexamethasone stimulates cell-associated and soluble chondroitin 4-sulfate proteoglycans, J. Biol. Chem. 266 (1991) 11237–11244.

[34] Camirand A., Marie V., Rabelo R., Silva J.E., Thiazolidinediones stimulate uncoupling protein-2 expression in cell lines representing white and brown adipose tissues and skeletal muscle, Endocrinology 139 (1998) 428–431.

[35] Carter-Su C., Smit L.S., Signaling via JAK tyrosine kinases: growth hormone receptor as a model system, Recent Prog. Horm. Res. 53 (1998) 61–83.

[36] Casimir D.A., Ntambi J.M., cAMP activates the expression of stearoyl-CoA desaturase gene 1 during early preadipocyte differentiation, J. Biol. Chem. 271 (1996) 29847–29853.

[37] Casimir D.A., Miller C.W., Ntambi J.M., Preadipocyte differentiation blocked by prostaglandin stimulation of prostanoid FP2 receptor in murine 3T3-L1 cells, Differentiation 60 (1996) 203–210.

[38] Castillo G., Brun R.P., Rosenfield J.K., Hauser S., Park C.W., Troy A.E., Wright M.E., Spiegelman B.M., An adipogenic cofactor bound by the differentiation domain of PPARgamma, EMBO J. 18 (1999) 3676–3687.

[39] Castro-Munozledo F., Marsch-Moreno M., Beltran-Langarica A., Kuri-Harcuch W., Commitment of adipocyte differentiation in 3T3 cells is inhibited by retinoic acid, and the expression of lipogenic enzymes is modulated through cytoskeleton stabilization, Differentiation 36 (1987) 211–219.

[40] Catalioto R.M., Gaillard D., Ailhaud G., Négrel R., Terminal differentiation of mouse preadipocyte cells: the mitogen-adipogenic role of growth hormone is mediated by the protein kinase C signaling pathway, Growth Factors 6 (1992) 255–264.

[41] Chapman A.B., Knight D.M., Dieckmann B.S., Ringold G.M., Analysis of gene expression during differentiation of adipogenic cells in culture and hormonal control of the developmental program, J. Biol. Chem. 259 (1984) 15548–15555.

[42] Chapman A.B., Knight D.M., Ringold G.M., Glucocorticoid regulation of adipocyte differentiation: hormonal triggering of the developmental program and induction of a differentiation-dependent gene, J. Cell. Biol. 101 (1985) 1227–1235.

[43] Chawla A., Lazar M.A., Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival, Proc. Natl. Acad. Sci. USA 91 (1994) 1786–1790.

[44] Chen N.X., White B.D., Hausman G.J. Glucocorticoid receptor binding in porcine preadipocytes during development, J. Anim. Sci. 73 (1995) 722–727.

[45] Chen N.X., Hausman G.J., Wright J.T., Influence of age and fetal hypophysectomy on porcine preadipocytes: insulin-like growth factor-I (IGF-I) response, receptor binding and IGF binding proteins secretion, Growth Dev. Aging 59 (1995) 193–206.

[46] Chen N.X., Hausman G.J., Wright J.T., Hormonal regulation of insulin-like growth factor binding proteins and insulin-like growth factor I (IGF-I) secretion in porcine stromal-vascular cultures, J. Anim. Sci. 74 (1996) 2369–2375.

[47] Chen H, Weng Y.C., Schatteman G.C., Sanders L., Christy R.J., Christy B.A., Expression of the dominant-negative regulator Id4 is induced during adipocyte differentiation, Biochem. Biophys. Res. Commun. 256 (1999) 614–619.

[48] Cianflone K., Maslowska M., Sniderman A.D., Acylation stimulating protein (ASP), an adipocyte autocrine: new directions, Cell Dev. Biol. 10 (1999) 31–41.

[49] Clark E.A., Brugge J.S., Integrins and signal transduction pathways: the road taken, Science 268 (1995) 233–238.

[50] Corin R.E., Guller S., Wu K.-Y., Sonenberg M., Growth hormone and adipose differentiation: growth hormone-induced antimitogenic state in 3T3-F442A preadipose cells, Proc. Natl. Acad. Sci. USA 87 (1990) 7507–7511.

[51] Cowherd R.M., Lyle R.E., McGehee R.E., Molecular regulation of adipocyte differentiation, Cell Dev. Biol. 10 (1999) 3–10.

[52] Crandall D.L., Hausman G.J., Kral J.G., A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives, Microcirculation 4 (1997) 211–232.

[53] Crandall D.L., Armellino D.C., Busler D.E., McHendry-Rinde B., Kral J.G., Angiotensin II receptors in human preadipocytes: role in cell cycle regulation, Endocrinology 140 (1999) 154–158.

[54] Crespo P., Mischak H., Gutkind J.S., Overexpression of mammalian protein kinase C-ζ does not affect the growth characteristics of NIH 3T3 cells, Biochem. Biophys. Res. Commun. 213 (1995) 266–272.

[55] Dace A., Sarkissian G., Schneider L., Martin-El Yazidi C., Bonne J., Margotat A., Planells R., Torresani J., Transient expression of c-erbA-beta1 messenger ribonucleic acid and beta1 thyroid hormone receptor early in adipogenesis of Ob17 cells, Endocrinology 140 (1999) 2983–2990.

[56] Dani C., Doglio A., Amri E.-Z., Bardon S., Fort P., Bertrand B., Grimaldi P., Ailhaud G., Cloning and regulation of a mRNA specifically expressed in the preadipose state, J. Biol. Chem. 264 (1989) 10119–10125.

[57] Dani C., Smith A.G., Dessolin S., Leroy P., Staccini L., Villageois P., Darimont C., Ailhaud G., Differentiation of embryonic stem cells into adipocytes in vitro, J. Cell. Sci. 110 (1997) 1279–1285.

[58] Darimont C., Vassaux G., Ailhaud G., Négrel R., Differentiation of preadipose cells: paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin-II, Endocrinology 135 (1994) 2030–2036.

[59] Deslex S., Négrel R., Vannier C., Etienne J., Ailhaud G., Differentiation of human adipocyte precursors in a chemically defined serum-free medium, Int. J. Obes. 10 (1986) 19–27.

[60] Deslex S., Négrel R., Ailhaud G., Development of a chemically defined serum-free medium for differentiation of rat adipose precursor cells, Exp. Cell. Res. 168 (1987) 15–30.

[61] Desvergne B., Petty K.J., Nikodem V.M., Functional characterization and receptor binding studies of the malic enzyme thyroid hormone response element, J. Biol. Chem. 266 (1991) 1008–1013.

[62] Diamond L., O'Brien T.G., Rovera G., Inhibition of adipose conversion of 3T3 fibroblasts by tumour promoters, Nature 269 (1977) 247–249.

[63] Dimaculangan D.D., Chawla A., Boak A., Kagan H.M., Lazar M.A., Retinoic acid prevents downregulation of RAS recision gene/lysyl oxidase early in adipocyte differentiation, Differentiation 58 (1994) 47–52.

[64] Doglio A., Dani C., Grimaldi P., Ailhaud G., Growth hormone regulation of the expression of differentiation-dependent genes in preadipocyte Ob1771 cells, Biochem. J. 238 (1986) 123–129.

[65] Doglio A., Dani C., Fredrikson G., Grimaldi P., Ailhaud G., Acute regulation of insulin-like growth factor-I gene expression by growth hormone during adipose cell differentiation, EMBO J. 6 (1987) 4011–4016.

[66] Doglio A., Dani C., Grimaldi P., Ailhaud G., Growth hormone stimulates *c-fos* gene expression by means of protein kinase C without increasing inositol lipid turnover, Proc. Natl. Acad. Sci. USA 86 (1989) 1148–1152.

[67] Donnelly L.E., Cryer A., Butterwith S.C., Comparison of the rates of proliferation of adipocyte precursor cells derived from two lines of chicken which differ in their rates of adipose tissue development, Br. Poult. Sci. 34 (1993) 187–193.

[68] Entenmann G., Hauner H., Relationship between replication and differentiation in cultured human adipocyte precursor cells, Am. J. Physiol. 270 (1996) C1011–C1016.

[69] Fève B., Moldes M., El Hadri K., Lasnier F., Pairault J., La différenciation adipocytaire: tout un programme, Médecine/Sciences 14 (1998) 848–857.

[70] Fiddler T.A., Smith L., Tapscott S.J., Thayer M.J., Amplification of MDM2 inhibits MyoD-mediated myogenesis, Mol. Cell. Biol. 16 (1996) 5048–5057.

[71] Fleming I., MacKenzie S.J., Vernon R.G., Anderson N.G., Houslay M.D., Kilgour E., Protein kinase C isoforms play differential roles in the regulation of adipocyte differentiation, Biochem. J. 333 (1998) 719–727.

[72] Fleury C., Neverova M., Collins S., Raimbault S., Champigny O., Levi-Meyrueis C., Bouillaud F., Seldin M.F., Surwit R.S., Ricquier D., Warden C.H., Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia, Nat. Genet. 15 (1997) 269–272.

[73] Flores-Delgado G., Marsch-Moreno M., Kuri-Harcuch W., Thyroid hormone stimulates adipocyte differentiation of 3T3 cells, Mol. Cell. Biochem. 76 (1987) 35–43.

[74] Font de Mora J., Porras A., Ahn N., Santos E., Mitogen-activated protein kinase activation is not necessary for, but antagonizes, 3T3-L1 adipocytic differentiation, Mol. Cell. Biol. 17 (1997) 6068–6075.

[75] Forgue-Lafitte M.-E., Van R.L., De Gasquet P., Rosselin G., The presence of epidermal growth factor receptors in cultured human adipocyte precursors, Scand. J. Clin. Lab. Invest. 42 (1982) 627–631.

[76] Frohnert B.I., Hui T.Y., Bernlohr D.A., Identification of a functional peroxisome proliferatior-response element in the murine fatty acid transport protein gene, J. Biol. Chem. 274 (1999) 3971–3977.

[77] Fukuda H., Iritani N., Sugimoto T., Ikeda H., Transcriptional regulation of fatty acid synthase gene by insulin/glucose, polyunsaturated fatty acid and leptin in hepatocytes and adipocytes in normal and genetically obese rats, Eur. J. Biochem. 260 (1999) 505–511.

[78] Gaben-Cogneville A.-M., Poussin B., Chamblier M-C., Forgue-Lafitte M-E., Rosselin G., Development of insulin and epidermal growth factor receptors during the differentiation of rat preadipocytes in primary culture, Biochim. Biophys. Acta 968 (1988) 231–238.

[79] Gaillard D., Négrel R., Lagarde M., Ailhaud G., Requirement and role of arachidonic acid in the differentiation of pre-adipose cells, Biochem. J. 257 (1989) 389–397.

[80] Gaillard D., Wabitsch M., Pipy B., Négrel R., Control of terminal differentiation of adipose precursor cells by glucocorticoids, J. Lipid. Res. 32 (1991) 569–579.

[81] Gaskins H.R., Kim J.-W., Hausman G.J., Decreases in local hormone biosynthesis and c-fos gene expression accompany differentiation of porcine preadipocytes, In Vitro Cell. Dev. Biol. 26 (1990) 1049–1056.

[82] Gaskins H.R., Kim J.-W., Wright J.T., Rund L.A., Hausman G.J., Regulation of insulin-like growth factor-I ribonucleic acid expression, polypeptide secretion, and binding protein activity by growth hormone in porcine preadipocyte cultures, Endocrinology 126 (1990) 622–630.

[83] Gharbi-Chihi J., Grimaldi P., Torresani J., Ailhaud G., Triiodothyronine and adipose conversion of OB_{17} preadipocytes: binding to high affinity sites and effects on fatty acid synthetizing and esterifying enzymes, J. Recept. Res. 2 (1981) 153–173.

[84] Gonzalez-Manchon C., Butta N., Ferrer M., Ayuso M.S., Parrilla R., Molecular cloning and functional characterization of the human cytosolic malic enzyme promoter: thyroid hormone responsiveness, DNA Cell Biol. 16 (1997) 533–544.

[85] Gonzalez-Manchon C., Ayuso M.S., Parrilla R., AP-1 and T3RE cis elements operate as a functional unit in the transcriptional control of the human malic enzyme gene, Gene 226 (1999) 111–119.

[86] Göttlicher M., Heck S., Herrlich P., Transcriptional cross-talk, the second mode of steroid hormone receptor action, J. Mol. Med. 76 (1998) 480–489.

[87] Green S., Promiscuous liaisons, Nature 361 (1993) 590–591.

[88] Green H., Kehinde O., Spontaneous heritable change leading to increased adipose conversion, Cell 7 (1976) 105–113.

[89] Green H., Morikawa M., Nixon T., A dual effector theory of growth-hormone action, Differentiation 29 (1985) 195–198.

[90] Grégoire F., Genart C., Hauser N., Remacle C., Glucocorticoids induce a drastic inhibition of proliferation and stimulate differentiation of adult rat fat cell precursors, Exp. Cell. Res. 196 (1991) 270–278.

[91] Grégoire F., De Broux N., Hauser N., Heremans H., van Damme J., Remacle C., Interferon-gamma and interleukin-1 beta inhibit adipoconversion in cultured rodent preadipocytes, J. Cell Physiol. 151 (1992) 300–309.

[92] Grégoire F.M., Johnson P.R., Greenwood M.R.C., Comparison of the adipoconversion of preadipocytes derived from lean and obese Zucker rats in serum-free cultures, Int. J. Obes. 19 (1995) 664–670.

[93] Grégoire F.M., Smas C.M., Sul H.S., Understanding adipocyte differentiation, Physiol. Rev. 78 (1998) 783–809.

[94] Grimaldi P.A., Teboul L., Gaillard D., Armengod A.V., Amri E.-Z., Long chain fatty acids as modulators of gene transcription in preadipose cells, Mol. Cell. Biochem. 192 (1999) 63–68.

[95] Guller S., Corin R.E., Mynarcik D.C., London B.M., Sonenberg M., Role of insulin in growth hormone-stimulated 3T3 cell adipogenesis, Endocrinology 122 (1988) 2084–2089.

[96] Guller S., Corin R.E., Wu K.-Y., Sonenberg M., Growth hormone-induced alteration of morphology and tubulin expression in 3T3 preadipose cells, Biochem. Biophys. Res. Commun. 163 (1989) 895–901.

[97] Guller S., Sonenberg M., Wu K.-Y., Szabo P., Corin R.E., Growth hormone-dependent events in the adipose differentiation of 3T3-F442A fibroblasts: modulation of macromolecular synthesis, Endocrinology 125 (1989) 2360–2367.

[98] Guller S., Corin R.E., Wu K.-Y., Sonenberg M., Up-regulation of vinculin expression in 3T3 preadipose cells by growth hormone, Endocrinology 129 (1991) 527–533.

[99] Hainque B., Moustaid N., Quignard-Boulange A., Ardouin B., Lavau M., Glucocorticoid binding during the differentiation of 3T3-F442A fibroblasts into adipocytes. A possible regulatory effect of insulin, Biochim. Biophys. Acta 931 (1987) 347–353.

[100] Hancock J.T., Cell signalling, Longman (Ed.), Essex-England, 1997.

[101] Haq R., Chytil F., Effect of retinoids on nuclear retinoic acid receptors mRNA in adipose tissue of retinol-deficient rats, J. Lipid. Res. 33 (1992) 381–384.

[102] Haraguchi K., Shimura H., Kawaguchi A., Ikeda M., Endo T., Onaya T., Effects of thyrotropin on the proliferation and differentiation of cultured rat preadipocytes, Thyroid 9 (1999) 613–619.

[103] Hauner H., Complete adipose differentiation of 3T3-L1 cells in a chemically defined medium: comparison to serum-containing culture conditions, Endocrinology 127 (1990) 865–872.

[104] Hauner H., Entenmann G., Regional variation of adipose differentiation in cultured stromal-vascular cells from the abdominal and femoral adipose tissue of obese woman, Int. J. Obes. 15 (1991) 121–126.

[105] Hauner H., Schmid P., Pfeiffer E.F., Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells, J. Clin. Endocrinol. Metab. 64 (1987) 832–835.

[106] Hauner H., Entenmann G., Wabitsch M., Gaillard D., Ailhaud G., Négrel R., Pfeiffer E.F., Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium, J. Clin. Invest. 84 (1989) 1663–1670.

[107] Hauner H., Röhrig K., Petruschke T., Effects of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) on human adipocyte development and function, Eur. J. Clin. Invest. 25 (1995) 90–96.

[108] Haupt Y., Maya R., Kazaz A., Oren M., Mdm2 promotes the rapid degradation of p53, Nature 387 (1997) 296–299.

[109] Hausman G.J., The influence of insulin, triiodothyronine (T_3) and insulin-like growth factor-I (IGF-1) on the differentiation of preadipocytes in serum-free cultures of pig stromalvascular cells, J. Anim. Sci. 67 (1989) 3136–3143.

[110] Hausman G.J., Responsiveness to adipogenic agents in stromal-vascular cultures derived from lean and preobese pig fetuses: an ontogeny study, J. Anim. Sci. 70 (1992) 106–114.

[111] Hausman G.J., The influence of dexamethasone and insulin on expression of CCAAT/enhancer binding protein isoforms during preadipocyte differentiation in porcine stromal-vscular cell cultures: evidence for very early expression of C/EBPalpha, J. Anim. Sci. 78 (2000) 1227–1235.

[112] Hausman G.J., Martin R.J., The influence of human growth hormone on preadipocyte development in serum-supplemented and serum-free cultures of stromal-vascular cells from pig adipose tissue, Domest. Anim. Endocrinol. 6 (1989) 331–337.

[113] Hausman G.J., Richardson R.L., Newly recruited and pre-existing preadipocytes in cultures of porcine stromal-vascular cells: morphology, expression of extracellular matrix components, and lipid accretion, J. Anim. Sci. 76 (1998) 48–60.

[114] Hausman G.J., Hausman D.B., Martin R.J., Biochemical and cytochemical studies of preadipocyte differentiation in serum-free culture of porcine stromal-vascular cells: interaction of dexamethasone and growth hormone, Acta Anat. 143 (1992) 322–329.

[115] Hausman G.J., Wright J.T., Richardson R.L., The influence of extracellular matrix substrata on preadipocyte development in serum-free cultures of stromal-vascular cells, J. Anim. Sci. 74 (1996) 2117–2128.

[116] Hausman D.B., Hausman G.J., Martin R.J., Endocrine regulation of fetal adipose tissue metabolism in the pig: interaction of porcine growth hormone and thyroxine, Obes. Res. 7 (1999) 76–82.

[117] Hertz R., Berman I., Keppler D., Bar-Tana J., Activation of gene transcription by prostacyclin analogues is mediated by the peroxisome-proliferators-activated receptor, Eur. J. Biochem. 235 (1996) 242–247.

[118] Hertzel A.V., Bernlohr D.A., Regulation of adipocyte gene expression by polyunsaturated fatty acids, Mol. Cell. Biochem. 188 (1998) 33–39.

[119] Himms-Hagen J., Brown adipose tissue thermogenesis: interdisciplinary studies, FASEB J. 4 (1990) 2890–2898.

[120] Ho I.-C., Kim J.H.-J., Rooney J.W., Spiegelman B.M., Glimcher L.H., A potential role for the nuclear factor of activated T cells family of

transcriptional regulatory proteins in adipogenesis, Proc. Natl. Acad. Sci. USA 95 (1998) 15537–15541.

[121] Hofmann J., The potential for isoenzyme-selective modulation of protein kinase C, FASEB J. 11 (1997) 649–669.

[122] Hu E., Liang P., Spiegelman B.M., AdipoQ is a novel adipose-specific gene dysregulated in obesity, J. Biol. Chem. 271 (1996) 10697–10703.

[123] Huang C., Freake H.C., Thyroid hormone regulates the acetyl-CoA carboxylase PI promoter, Biochem. Biophys. Res. Commun. 249 (1998) 704–708.

[124] Hyman B.T., Stoll L.L., Spector A.A., Prostaglandin production by 3T3-L1 cells in culture, Biochim. Biophys. Acta 713 (1982) 375–385.

[125] Ignotz R.A., Massagué J., Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix, J. Biol. Chem. 261 (1986) 4337–4345.

[126] Imagawa M., Tsuchiya T., Nishihara T., Identification of inducible genes at the early stage of adipocyte differentiation of 3T3-L1 cells, Biochem. Biophys. Res. Commun. 254 (1999) 299–305.

[127] Janknecht R., Hunter T., A growing coactivator network, Nature 383 (1996) 22–23.

[128] Jiang H.-P., Harris S.E., Serrero G., Molecular cloning of a differentiation-related mRNA in the adipogenic cell line 1246, Cell Growth Differ. 3 (1992) 21–30.

[129] Jiang M.S., Tang Q.Q., McLenithan J., Geiman D., Shillinglaw W., Henzel W.J., Lane M.D., Derepression of the C/EBPα gene during adipogenesis: identification of AP-2alpha as a repressor, Proc. Natl. Acad. Sci. USA 95 (1998) 3467–3471.

[130] Kamei Y., Kawada T., Kazuki R., Sugimoto E., Retinoic acid receptor γ2 gene expression is upregulated by retinoic acid in 3T3-L1 preadipocytes, Biochem. J. 293 (1993) 807–812.

[131] Karlsson C., Lindell K., Ottosson M., Sjostrom L., Carlsson B., Carlsson L.M., Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II, J. Clin. Endocrinol. Metab. 83 (1998) 3925–3929.

[132] Kawada T., Kamei Y., Sugimoto E., The possibility of active form of vitamins A and D as suppressors on adipocyte development via ligand-dependent transcriptional regulators, Int. J. Obes. Relat. Metab. Disord. 20 (Suppl. 3) (1996) S52–S57.

[133] Keay S., Grossberg S.E., Interferon inhibits the conversion of 3T3-L1 mouse fibroblasts into adipocytes, Proc. Natl. Acad. Sci. USA 77 (1980) 4099–4103.

[134] Keller H., Dreyer C., Medin J., Mahfoudi A., Ozato K., Wahli W., Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers, Proc. Natl. Acad. Sci. USA 90 (1993) 2160–2164.

[135] Kim J.B., Spiegelman B.M., ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism, Genes Dev. 10 (1996) 1096–1107.

[136] Kim J.B., Sarraf P., Wright M., Yao K.M., Mueller E., Solanes G., Lowell B.B., Spiegelman B.M., Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1, J. Clin. Invest. 101 (1998) 1–9.

[137] Kim H.S., Hausman G.J., Hausman D.B., Martin R.J., Dean R.G., The expression of peroxisome proliferator-activated receptor gamma in pig fetal tissue and primary stromal-vascular cultures, Obes. Res. 8 (2000) 83–88.

[138] King A.P.J., Carter-Su C., Dexamethasone-induced antagonism of growth hormone (GH) action by down-regulation of GH binding in 3T3-F442A fibroblasts, Endocrinology 136 (1995) 4796–4803.

[139] Kirkland J.L., Hollenberg C.H., Gillon W.S., Age, anatomic site, and the replication and differentiation of adipocyte precursors, Am. J. Physiol. 258 (1990) C206–C210.

[140] Kjeldsen T., Andersen A.S., Wiberg F.C., Rasmussen J.S., Schäffer L., Balschmidt P., Moller K.B., Moller N.P.H., The ligand specificities of the insulin receptor and the insulin-like growth factor I receptor reside in different regions of a common binding site, Proc. Natl. Acad. Sci. USA 88 (1991) 4404–4408.

[141] Kliewer S.A., Umesono K., Mangelsdorf D.J., Evans R.M., Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling, Nature 355 (1992) 446–449.

[142] Kliewer S.A., Lenhard J.M., Willson T.M., Patel I., Morris D.C., Lehmann J.M., A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation, Cell 83 (1995) 813–819.

[143] Konieczny S.F., Emerson C.P., 5-azacytidine induction of stable mesodermal stem cell lineages from 10T1/2 cells: evidence for regulatory genes controlling determination, Cell 38 (1984) 791–800.

[144] Kubbutat M.H.G., Jones S.N., Vousden K.H., Regulation of p53 stability by Mdm2, Nature 387 (1997) 299–303.

[145] Kuri-Harcuch W., Differentiation of 3T3-F442A cells into adipocytes is inhibited by retinoic acid, Differentiation 23 (1982) 164–169.

[146] La Thangue N.B., DP and E2F proteins: components of a heterodimeric transcription factor implicated in cell cycle control, Curr. Opin. Cell Biol. 6 (1994) 443–450.

[147] Lacasa D., Agli B., Giudicelli Y., Zeta PKC in rat preadipocytes: modulation by insulin and serum mitogenic factors and possible role in adipogenesis, Biochem. Biophys. Res. Commun. 217 (1995) 123–130.

[148] Latimer A.M., Hausman G.J., McCusker R.H., Buonomo F.C., The effects of thyroxine on serum and tissue concentrations of insulin-like growth factors (IGF-I and -II) and IGF-binding proteins in the fetal pig, Endocrinology 133 (1993) 1312–1319.

[149] Lebret B., Mourot J., Caractéristiques et qualité des tissus adipeux chez le porc. Facteurs de variations non génétiques, INRA Prod. Anim. 11 (1998) 131–143.

[150] Leid M., Kastner P., Chambon P., Multiplicity generates diversity in the retinoic acid signalling pathways, Trends Biochem. Sci. 17 (1992) 427–433.

[151] Lelièvre S., Weaver V.M., Bissell M.J., Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: a model of gene regulation, Recent Prog. Horm. Res. 51 (1996) 417–432.

[152] Litthauer D., Serrero G., The primary culture of mouse adipocyte precursor cells in defined medium, Comp. Biochem. Physiol. A 101 (1992) 59–64.

[153] Loftus T.M., Introduction: fat metabolism and adipose homeostasis, Cell Dev. Biol. 10 (1999) 1–2.

[154] Luetteke N.C., Lee D.C., Palmiter R.D., Brinster R.L., Sandgren E.P., Regulation of fat and muscle development by transforming growth factor α in transgenic mice and in cultured cells, Cell Growth Differ. 4 (1993) 203–213.

[155] Lyle R.E., Richon V.M., McGehee R.E., TNFα disrupts mitotic clonal expansion and regulation of retinoblastoma proteins p130 and p107 during 3T3-L1 adipocyte differentiation, Biochem. Biophys. Res. Commun. 247 (1998) 373–378.

[156] Mandrup S., Lane M.D., Regulating adipogenesis, J. Biol. Chem. 272 (1997) 5367–5370.

[157] Massagué J., TGF-β signal transduction, Annu. Rev. Biochem. 67 (1998) 753–791.

[158] May J.M., de Haen C., The insulin-like effect of hydrogen peroxide on pathways of lipid synthesis in rat adipocytes, J. Biol. Chem. 254 (1979) 9017–9021.

[159] McGowan K., DeVente J., Carey J.O., Ways D.K., Pekala P.H., Protein kinase C isoform expression during the differentiation of 3T3-L1 preadipocytes: loss of protein kinase C-α isoform correlates with loss of phorbol 12-myristate 13-acetate activation of nuclear factor κB and acquisition of the adipocyte phenotype, J. Cell Physiol. 167 (1996) 113–120.

[160] McInnes C., Sykes B.D., Growth factor receptors: structure, mechanism, and drug discovery, Biopolymers 43 (1998) 339–366.

[161] Mellor H., Parker P.J., The extended protein kinase C superfamily, Biochem. J. 332 (1998) 281–292.

[162] Moldes M., Fève B., Pairault J., Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage. Differentiation-dependent expression, regulation, and identification as semicarbazide-sensitive amine oxidase, J. Biol. Chem. 274 (1999) 9515–9523.

[163] Morrison R.F., Farmer S.R., Role of PPARγ in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18 (INK4c) and p21 (waf1/Cip1), during adipogenesis, J. Biol. Chem. 274 (1999) 17088–17097.

[164] Moutoussamy S., Kelly P.A., Finidori J., Growth-hormone-receptor and cytokine-receptor-family signaling, Eur. J. Biochem. 255 (1998) 1–11.

[165] Nakaigawa N., Hirai S.-I., Mizuno K., Shuin T., Hosaka M., Ohno S., Differential effects of overexpression of PKCα and PKCδ/ε on cellular E2F activity in late G1 phase, Biochem. Biophys. Res. Commun. 222 (1996) 95–100.

[166] Navre M., Ringold G.M., A growth factor-repressible gene associated with protein kinase C-mediated inhibition of adipocyte differentiation, J. Cell Biol. 107 (1988) 279–286.

[167] Navre M., Ringold G.M., Differential effects of fibroblast growth factor and tumor promoters on the initiation and maintenance of adipocyte differentiation, J. Cell Biol. 109 (1989) 1857–1863.

[168] Négrel R., Grimaldi P., Ailhaud G., Establishment of preadipocyte clonal line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic hormones, Proc. Natl. Acad. Sci. USA 75 (1978) 6054–6058.

[169] Négrel R., Grimaldi P., Ailhaud G., Differentiation of Ob17 preadipocytes to adipocytes effect of prostaglandin $F_{2\alpha}$ and relationship to prostaglandin synthesis, Biochim. Biophys. Acta 666 (1981) 15–24.

[170] Négrel R., Gaillard D., Ailhaud G., Prostacyclin as a potent effector of adipose-cell differentiation, Biochem. J. 257 (1989) 399–405.

[171] Nikodem V.M., Magnuson M.A., Dozin B., Morioka H., Coding nucleotide sequence of rat malic enzyme mRNA and tissue specific regulation by thyroid hormone, Endocr. Res. 15 (1989) 547–564.

[172] Nishizuka Y., The role of protein kinase C in cell surface signal transduction and tumour promotion, Nature 308 (1984) 693–698.

[173] Ntambi J.M., Sessler A.M., Takova T., A model cell line to study regulation of stearoyl-CoA desaturase gene 1 expression by insulin and polyunsaturated fatty acids, Biochem. Biophys. Res. Commun. 220 (1996) 990–995.

[174] Ohyama M., Matsuda K., Torii S., Matsui T., Yano H., Kawada T. Ishihara T., The interaction

between vitamin A and thiazolidinedione on bovine adipocyte differentiation in primary culture, J. Anim. Sci. 76 (1998) 61–65.

[175] Okamoto Y., Arita Y., Nishida M., Muraguchi M., Ouchi N., Takahashi M., Igura T., Inui Y., Kihara S., Nakamura T., Yamashita S., Miyagawa J., Funahashi T., Matsuzawa Y., An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls, Horm. Metab. Res. 32 (2000) 47–50.

[176] Pairault J., Lasnier F., Dihydrocytochalasin B promotes adipose conversion of 3T3 cells, Biol. Cell. 61 (1987) 149–154.

[177] Patel Y.M., Lane M.D., Mitotic clonal expansion during preadipocyte differentiation: calpain-mediated turnover of p27, J. Biol. Chem. 275 (2000) 17653–17660.

[178] Petruschke T.H., Hauner H., Tumor necrosis factor-α prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells, J. Clin. Endocrinol. Metab. 76 (1993) 742–747.

[179] Petruschke T., Röhrig K., Hauner H., Transforming growth factor beta (TGF-beta) inhibits the differentiation of human adipocyte precursor cells in primary culture, Int. J. Obes. 18 (1994) 532–536.

[180] Petty K.J., Desvergne B., Mitsuhashi T., Nikodem V.M., Identification of a thyroid hormone response element in the malic enzyme gene, J. Biol. Chem. 265 (1990) 7395–7400.

[181] Reichert M., Eick D., Analysis of cell cycle arrest in adipocyte differentiation, Oncogene 18 (1999) 459–466.

[182] Reyne Y., Nouguès J., Dulor J.-P., Differentiation of rabbit adipocyte precursor cells in a serum-free medium, In Vitro Cell. Dev. Biol. 25 (1989) 747–752.

[183] Rhee S.G., Redox signaling: hydrogen peroxide as intracellular messenger, Exp. Mol. Med. 31 (1999) 53–59.

[184] Richardson R.L., Campion D.R., Hausman G.J., Wright J.T., Transforming growth factor type β (TGF-β) and adipogenesis in pigs, J. Anim. Sci. 67 (1989) 2171–2180.

[185] Richardson R.L., Hausman G.J., Gaskins H.R., Effect of transforming growth factor beta on insulin-like growth factor 1- and dexamethasone-induced proliferation and differentiation in primary cultures of pig preadipocytes, Acta Anat. 145 (1992) 321–326.

[186] Richardson R.L., Hausman G.J., Wright J.T., Growth factor regulation of insulin-like growth factor (IGF) binding proteins (IGFBP) and preadipocyte differentiation in porcine stromal-vascular cell cultures, Growth Dev. Aging 62 (1998) 3–12.

[187] Richon V.M., Lyle R.E., McGehee R.E., Regulation and expression of retinoblastoma proteins p107 and p130 during 3T3-L1 adipocyte differentiation, J. Biol. Chem. 272 (1997) 10117–10124.

[188] Ricquier D., Casteilla L., Bouillaud F., Molecular studies of the uncoupling protein, FASEB J. 5 (1991) 2237–2242.

[189] Rubin C.S., Hirsch A., Fung C., Rosen O.M., Development of hormone receptors and hormonal responsiveness in vitro, J. Biol. Chem. 253 (1978) 7570–7578.

[190] Safonova I., Darimont C., Amri E.-Z., Grimaldi P., Ailhaud G., Reichert U., Shroot B., Retinoids are positive effectors of adipose cell differentiation, Mol. Cell. Endocrinol. 104 (1994) 201–211.

[191] Safonova I., Reichert U., Shroot B., Ailhaud G., Grimaldi P., Fatty acids and retinoids act synergistically on adipose cell differentiation, Biochem. Biophys. Res. Commun. 204 (1994) 498–504.

[192] Sato M., Hiragun A., Mitsui H., Preadipocytes possess cellular retinoid binding proteins and their differentiation is inhibited by retinoids, Biochem. Biophys. Res. Commun. 95 (1980) 1839–1845.

[193] Satory D.L., Smith S.B., Conjugated linoleic acid inhibits proliferation but stimulates lipid filling of murine 3T3-L1 preadipocytes, J. Nutr. 129 (1999) 92–97.

[194] Schaffer J.E., Lodish H.F., Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein, Cell 79 (1994) 427–436.

[195] Schmidt W., Poll-Jördan G., Löffler G., Adipose conversion of 3T3-L1 cells in a serum-free culture system depends on epidermal growth factor, insulin-like growth factor I, corticosterone, and cyclic AMP, J. Biol. Chem. 265 (1990) 15489–15495.

[196] Schwarz E.J., Reginato M.J., Shao D., Krakow S.L., Lazar M.A., Retinoic acid blocks adipogenesis by inhibiting C/EBPbeta-mediated transcription, Mol. Cell. Biol. 17 (1997) 1552–1561.

[197] Seedorf K., Intracellular signaling by growth factors, Metabolism 44 (Suppl. 4) (1995) 24–32.

[198] Serrero G., EGF inhibits the differentiation of adipocyte precursors in primary cultures, Biochem. Biophys. Res. Commun. 146 (1987) 194–202.

[199] Serrero G., Lepak N., Endocrine and paracrine negative regulators of adipose differentiation, Int. J. Obes. 20 (Suppl. 3) (1996) S58–S64.

[200] Serrero G., Mills D., Differentiation of newborn rat adipocyte precursors in defined serum-free medium, In Vitro Cell. Dev. Biol. 23 (1987) 63–66.

[201] Serrero G., Mills D., Physiological role of epidermal growth factor on adipose tissue development in vivo, Proc. Natl. Acad. Sci. USA 88 (1991) 3912–3916.

[202] Serrero G., Lepak N.M., Goodrich S.P., Paracrine regulation of adipose differentiation by arachidonate metabolites-prostaglandin-$F_{2\alpha}$ inhibits early and late markers of differentiation in the adipogenic cell line-1246, Endocrinology 131 (1992) 2545–2551.

[203] Serrero G., Lepak N.M., Goodrich S.P., Prostaglandin F2alpha inhibits the differentiation of adipocyte precursors in primary culture, Biochem. Biophys. Res. Commun. 183 (1992) 438–442.

[204] Serrero G., Lepak N.M., Hayashi J., Goodrich S.P., Impaired epidermal growth factor production in genetically obese ob/ob mice, Am. J. Physiol. 264 (1993) E800–E803.

[205] Sethi J.K., Hotamisligil G.S., The role of TNFα in adipocyte metabolism, Cell Dev. Biol. 10 (1999) 19–29.

[206] Sfeir Z., Ibrahimi A., Amri E., Grimaldi P., Abumrad N., CD36 antisense expression in 3T3-F442A preadipocytes, Mol. Cell. Biochem. 192 (1999) 3–8.

[207] Shimizu Y., Shimizu N., Fujiki H., Sugimura T., Distinct inhibitory effects of dihydroteleocidin B and the phorbol ester tumor promotors on the adipocyte differentiation of 3T3-L1 cells, Cancer Res. 43 (1983) 4974–4979.

[208] Shinohara O., Murata Y.-I., Shimizu M., Endothelin-1 suppression of rat adipocyte precursor cell differentiation in serum-free culture, Endocrinology 130 (1992) 2031–2036.

[209] Shinohara O., Murata Y-I., Kubota C., Shinagawa T., Protein kinase C inhibitors enhance differentiation of rat adipocyte precursor cells in serum-free culture, Biochem. Med. Metab. Biol. 51 (1994) 85–90.

[210] Shugart E.C., Leveson A.S., Constance C.M., Umek R.M., Differential expression of gas and gadd genes at distinct growth arrest points during adipocyte development, Cell Growth Differ. 6 (1995) 1541–1547.

[211] Sinha D., Addya S., Murer E., Boden G., 15-deoxy-delta(12, 14) prostaglandin J2: a putative endogenous promoter of adipogenesis suppresses the ob gene, Metabolism 48 (1999) 786–791.

[212] Smas C.M., Sul H.S., Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation, Cell 73 (1993) 725–734.

[213] Smas C.M., Sul H.S., Characterization of Pref-1 and its inhibitory role in adipocyte differentiation, Int. J. Obes. 20 (Suppl. 3) (1996) S65–S72.

[214] Smas C.M., Chen L., Zhao L., Latasa M.J., Sul H.S., Transcriptional repression of Pref-1 by glucocorticoids promotes 3T3-L1 adipocyte differentiation, J. Biol. Chem. 274 (1999) 12632–12641.

[215] Smith P.J., Wise L.S., Berkowitz R., Wan C., Rubin C.S., Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes, J. Biol. Chem. 263 (1988) 9402–9408.

[216] Soret B., Lee H.J., Finley E., Lee S.C., Vernon R.G., Regulation of differentiation of sheep subcutaneous and abdominal preadipocytes in culture, J. Endocrinol. 161 (1999) 517–524.

[217] Sorisky A., From preadipocyte to adipocyte: differentiation-directed signals of insulin from the cell surface to the nucleus, Crit. Rev. Clin. Lab. Sci. 36 (1999) 1–34.

[218] Sparks R.L., Scott R.E., Transforming growth factor type β is a specific inhibitor of 3T3 T mesenchymal stem cell differentiation, Exp. Cell. Res. 165 (1986) 345–352.

[219] Sparks R.L., Allen B.J., Strauss E.E., TGF-β blocks early but not late differentiation-specific gene expression and morphologic differentiation of 3T3 T proadipocytes, J. Cell. Physiol. 150 (1992) 568–577.

[220] Sparks R.L., Strauss E.E., Manga A.V., Regulation of differentiation and protein kinase C expression in 3T3 T proadipocytes: effects of TGF-β and transformation, Cell. Prolif. 27 (1994) 139–151.

[221] Spiegelman B.M., Ginty C.A., Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes, Cell 35 (1983) 657–666.

[222] Spiegelman B.M., Green H., Cyclic AMP-mediated control of lipogenic enzyme synthesis during adipose differentiation of 3T3 cells, Cell 24 (1981) 503–510.

[223] Staiger H., Löffler G., The role of PDGF-dependent suppression of apoptosis in differentiating 3T3-L1 preadipocytes, Eur. J. Cell Biol. 77 (1998) 220–227.

[224] Stone R.L., Bernlohr D.A., The molecular basis for inhibition of adipose conversion of murine 3T3-L1 cells by retinoic acid, Differentiation 45 (1990) 119–127.

[225] Strobel A., Siquier K., Zilberfarb V., Strosberg A.D., Issad T., Effect of thiazolidinediones on expression of UCP2 and adipocyte markers in human PAZ6 adipocytes, Diabetologia 42 (1999) 527–533.

[226] Suryawan A., Hu C.Y., Effect of retinoic acid on differentiation of cultured pig preadipocytes, J. Anim. Sci. 75 (1997) 112–117.

[227] Suryawan A., Swanson L.V., Hu C.Y., Insulin and hydrocortisone, but not triiodothyronine, are required for the differentiation of pig preadipocytes in primary culture, J. Anim. Sci. 75 (1997) 105–111.

[228] Tabor D.E., Kim J.B., Spiegelman B.M., Edwards P.A., Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2, J. Biol. Chem. 274 (1999) 20603–20610.

[229] Tang Q.Q., Jiang M.S., Lane M.D., Repression of transcription mediated by dual elements in the CCAAT/enhancer binding protein alpha gene, Proc. Natl. Acad. Sci. USA 94 (1997) 13571–13575.

[230] Tang Q.Q., Jiang M.S., Lane M.D., Repressive effect of Sp1 on the C/EBPalpha gene promoter: role in adipocyte differentiation, Mol Cell Biol. 19 (1999) 4855–4865.

[231] Tapscott S.J., Davis R.L., Thayer M.J., Cheng P., Weintraub H., Lassar A.B., MYOD1: a nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts, Science 242 (1988) 405–411.

[232] Tatsis-Kotsidis I., Erlanger B.F., Initiation of a process of differentiation by stable transfection of Ob17 preadipocytes with the cDNA of human A1 adenosine receptor, Biochem. Pharmacol. 58 (1999) 167–170.

[233] Taylor S.M., Jones P.A., Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine, Cell 17 (1979) 771–779.

[234] Téboul M., Bismuth J., Gharbi-Chihi J., Vallette A., Bonne J., Ghiringhelli O., Torresani J., Retinoic acid decreases nuclear triiodothyronine receptor expression and impairs an early step of adipose differentiation in the thyroid hormone-sensitive mouse Ob17 preadipocyte cell line, Endocrinology 130 (1992) 1475–1482.

[235] Timchenko N.A., Wilde M., Nakanishi M., Smith J.R., Darlington G.J., CCAAT/enhancer-binding protein α (C/EBPα) inhibits cell proliferation through the p21 (WAF-1/CIP-1/SDI-1) protein, Genes Dev. 10 (1996) 804–815.

[236] Toker A., Signaling through protein kinase C, Front. Biosci. 3 (1998) d1134–d1147.

[237] Tontonoz P., Kim J.B., Graves R.A., Spiegelman B.M., ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation, Mol. Cell. Biol. 13 (1993) 4753–4759.

[238] Tontonoz P., Hu E., Spiegelman B.M., Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor, Cell 79 (1994) 1147–1156.

[239] Tontonoz P., Hu E., Graves R.A., Budavari A.I., Spiegelman B.M., mPPARγ2: tissue-specific regulator of an adipocyte enhancer, Genes Dev. 8 (1994) 1224–1234.

[240] Torti F.M., Torti S.V., Larrick J.W., Ringold G.M., Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta, J. Cell Biol. 108 (1989) 1105–1113.

[241] Ueda H., Ishida Y., Taniguchi H., Involvement of α- and β-PKC in the differentiation of 3T3-L1 cells, FEBS Lett. 292 (1991) 57–60.

[242] Ueda H., Ishida Y., Taniguchi H., Possible involvement of β-PKC rather than that of α-PKC in differentiation of 3T3-L1 cells to adipocytes, Eur. J. Clin. Invest. 23 (1993) 382–384.

[243] van der Vusse G.J., Glatz J.F.C., Stam H.C.G., Reneman R.S., Fatty acid homeostasis in the normoxic and ischemic heart, Phys. Rev. 72 (1992) 881–901.

[244] van Nieuwenhoven F.A., van der Vusse G.J., Glatz J.F.C., Membrane-associated and cytoplasmic fatty acid-binding proteins, Lipids 31 (1996) S223–S227.

[245] Vassaux G., Gaillard D., Ailhaud G., Négrel R., Prostacyclin is a specific effector of adipose cell differentiation, J. Biol. Chem. 267 (1992) 11092–11097.

[246] Vassaux G., Gaillard D., Darimont C., Ailhaud G., Négrel R., Differential response of preadipocytes and adipocytes to prostacyclin and prostaglandin E2: physiological implications, Endocrinology 131 (1992) 2393–2398.

[247] Vassaux G., Négrel R., Ailhaud G., Gaillard D., Proliferation and differentiation of rat adipose precursor cells in chemically defined medium: differential action of anti-adipogenic agents, J. Cell. Physiol. 161 (1994) 249–256.

[248] Vidal-Puig A., Solanes G., Grujic D., Flier J.S., Lowell B.B., UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue, Biochem. Biophys. Res. Commun. 235 (1997) 79–82.

[249] Wabitsch M., Hauner H., Heinze E., Teller W.M., The role of growth hormone/insulin-like growth factors in adipocyte differentiation, Metabolism 44 (Suppl. 4) (1995) 45–49.

[250] Wang H., Scott R.E., Inhibition of distinct steps in the adipocyte differentiation pathway in 3T3 T mesenchymal stem cells by dimethyl sulfoxide (DMSO), Cell. Prolif. 26 (1993) 55–66.

[251] Waters K.M., Miller C.W., Ntambi J.M., Localization of a negative thyroid hormone-response region in hepatic stearoyl-CoA desaturase gene 1, Biochem. Biophys. Res. Commun. 233 (1997) 838–843.

[252] Waters K.M., Miller C.W., Ntambi J.M., Localization of a polyunsaturated fatty acid response region in stearoyl-CoA desaturase gene 1, Biochim. Biophys. Acta 1349 (1997) 33–42.

[253] Weigel N.L., Zhang Y., Ligand-independent activation of steroid hormone receptors, J. Mol. Med. 76 (1998) 469–479.

[254] Weiner F.R., Shah A., Smith P., Rubin C.S., Zern M.A., Regulation of collagen gene expression in 3T3-L1 cells. Effects of adipocyte differentiation and tumor necrosis factor α, Biochemistry 28 (1989) 4094–4099.

[255] Wiederer O., Löffler G., Hormonal regulation of the differentiation of rat adipocyte precursor cells in primary culture, J. Lipid Res. 28 (1987) 649–658.

[256] Wolf G., Fatty acids bind directly to and activate peroxisome proliferator-activated receptors α and γ, Nutr. Rev. 56 (1998) 61–63.

[257] Wolf G., The molecular mechanism of the stimulation of adipocyte differentiation by a glucocorticoid, Nutr. Rev. 57 (1999) 324–326.

[258] Wrann M., Fox C.F., Ross R., Modulation of epidermal growth factor receptors on 3T3 cells by platelet-derived growth factor, Science 210 (1980) 1363–1365.

[259] Wright J.T., Hausman G.J., Insulin-like growth factor-1 (IGF-1)-induced stimulation of porcine preadipocyte replication, In Vitro Cell. Dev. Biol. 31 (1995) 404–408.

[260] Wu Z., Bucher N.L., Farmer S.R., Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids, Mol. Cell Biol. 16 (1996) 4128–4136.

[261] Xing H., Northrop J.P., Grove J.R., Kilpatrick K.E., Su J.L., Ringold G.M., TNF alpha-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPARgamma without effect on Pref-1 expression, Endocrinology 138 (1997) 2776–2783.

[262] Xue J.C., Schwarz E.J., Chawla A., Lazar M.A., Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARgamma, Mol. Cell. Biol. 16 (1996) 1567–1575.

[263] Yamasaki M., Emoto H., Konishi M., Mikami T., Ohuchi H., Nakao K., Itoh N., FGF-10 is a growth factor for preadipocytes in white adipose tissue, Biochem. Biophys. Res. Commun. 258 (1999) 109–112.

[264] Yang V.W., Christy R.J., Cook J.S., Kelly T.J., Lane M.D., Mechanism of regulation of the 422(aP2) gene by cAMP during preadipocyte differentiation, Proc. Natl. Acad. Sci. USA 86 (1989) 3629–3633.

[265] Yarwood S.J., Kilgour E., Anderson N.G., Cyclic AMP potentiates growth hormone-dependent differentiation of 3T3-F442A preadipocytes: possible involvement of the transcription factor CREB, Mol. Cell. Endocrinol. 138 (1998) 41–50.

[266] Yarwood S.J., Sale E.M., Sale G.J., Houslay M.D., Kilgour E., Anderson N.G., Growth hormone-dependent differentiation of 3T3-F442A preadipocytes requires Janus kinase/signal transducer and activator of transcription but not mitogen-activated protein kianse or p70 S6 kinase signaling, J. Biol. Chem. 274 (1999) 8662–8668.

[267] Yeh W-C., Cao Z., Classon M., McKnight S.L., Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins, Genes Dev. 9 (1995) 168–181.

[268] Yen P.M., Darling D.S., Chin W.W., Basal and thyroid hormone receptor auxiliary protein-enhanced binding of thyroid hormone receptor isoforms to native thyroid hormone response element, Endocrinology 129 (1991) 3331–3336.

[269] Yu Z.K., Wright J.T., Hausman G.J., Preadipocyte recruitment in stromal vascular cultures after depletion of committed preadipocytes by immunocytotoxicity, Obes. Res. 5 (1997) 9–15.

[270] Yun K., Scott R.E., Biological mechanisms of phorbol myristate acetate-induced inhibition of proadipocyte differentiation, Cancer Res. 43 (1983) 88–96.

[271] Zangani D., Darcy K.M., Masso-Welch P.A., Bellamy E.S., Desole M.S., Ip M.M., Multiple differentiation pathways of rat mammary stromal cells in vitro: acquisition of a fibroblast, adipocyte or endothelial phenotype is dependent on hormonal and extracellular matrix stimulation, Differentiation 64 (1999) 91–101.

[272] Zezulak K.M., Green H., The generation of insulin-like growth factor-1-sensitive cells by growth hormone action, Science 233 (1986) 551–553.

[273] Zhang X.-K., Hoffmann B., Tran P.B.-V., Graupner G., Pfahl M., Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors, Nature 355 (1992) 441–446.

[274] Zhang B., Berger J., Zhou G., Elbrecht A., Biswas S., White-Carrington S., Szalkowski D., Moller D.E., Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor γ, J. Biol. Chem. 271 (1996) 31771–31774.