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Is there a single mechanism for fatty acid regulation of gene transcription?

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Abstract

Besides their role as energetic molecules, fatty acids (FAs) also act as signals involved in regulating gene expression. This review focuses on a few examples of FA regulation. The hepatic lipogenic enzyme, fatty acid synthase (FAS) is negatively regulated by polyunsaturated FAs (PUFAs) which suppress sterol regulatory element-binding protein 1 (SREBP 1) gene expression and nuclear content in hepatocytes, thereby reducing *FAS* gene transcription. It was proposed recently that this reduction in SREBP 1 was the result of a PUFA-induced antagonism of ligand-dependent activation of the liver X nuclear receptor (LXR), known to be an inducer of the *SREBP 1* gene. In contrast, several genes are turned on by long-chain (LCFAs) and nonmetabolized FAs in a physiologically relevant manner. These include the acyl-CoA oxidase (AOX), the liver carnitine palmitoyltransferase 1 (L-CPT 1) and the liver fatty acid binding protein (L-FABP). While induction of *AOX* gene transcription appears to be PPARα-dependent, that of the *L-CPT 1* gene seems disconnected from PPAR activation. Results obtained in preadipocytes and in intestine cells are in support of a key role played by the β/δ isoform of PPAR in LCFA induction of the *FABP* gene. Transcription of the phosphoenolpyruvate carboxykinase (PEPCK) gene is stimulated by unsaturated and nonmetabolized LCFAs specifically in adipocytes. Our results reported here support the notion that the mechanisms by which PPARγ activators and FAs induce transcription of the *PEPCK* gene are distinct. Altogether these data argue that several FA effects are PPAR-independent. Evidences suggesting that other transcription factors might be involved are debated. It seems now clear that depending upon the cell-specific context and the target gene, FAs can take very different routes to alter transcription.

Keywords: Fatty acid; Gene; Transcription factor; Peroxisome proliferator activated receptor; Phosphoenolpyruvate carboxykinase; Adipocyte

1. Introduction

Most of the FA-regulated genes encode proteins playing a role in FA transport or metabolism (Fig. 1). Genes can be turned on or off by FAs. The corresponding change in the amount of specific proteins is an adaptive process that the cells develop in response to variations in FA concentration in the vicinity of the target tissue. Circulating nonesterified FAs (NEFAs) arise from white adipose tissue (WAT) as a consequence of lipolysis from the stored triacylglycerols during periods of starvation. The major proportion of blood NEFAs is bound to serum albumin while a much weaker amount remains unbound and is named free FA (FFA). NEFAs are cleared from the blood and oxidized in peripheral tissues, among which muscles are probably the most active. In lipogenic tissues like liver and WAT, FAs can be synthesized *de novo* (lipogenesis) from glucose following glycolysis. Liver, WAT and muscle are therefore major targets for FA regulation of gene expression. Inside

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Abbreviations: AOX, acyl-CoA oxidase; ALBP-aP2, adipocyte lipid binding protein; BSA, bovine serum albumin; CAT, chloramphenicol acetyl transferase; CPT 1, carnitine palmitoyltransferase 1; DHA, docosahexaenoic acid; DR1, direct repeat 1; DRB, 5,6-dichloro-1β-ribofuranosyl benzimidazole; FA, fatty acid; FAS, fatty acid synthase; HNF4, hepatic nuclear factor 4; L-PK, liver-type pyruvate kinase; LCFA, long-chain fatty acid; L-FABP, liver fatty acid binding protein; LXR, liver X receptor; NEFA, nonesterified fatty acid; Rosi, rosiglitazone; PEPCK, cytosolic phosphoenolpyruvate carboxykinase (EC 4.1.1.32); PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; PUFA, polyunsaturated fatty acid; RXR, retinoid X receptor; SREBP 1, sterol regulatory element-binding protein 1; SRE, SREBP response element; TF, transcription factor; Type II-DM, type II diabetes melitus; TZD, thiazolidinedione.

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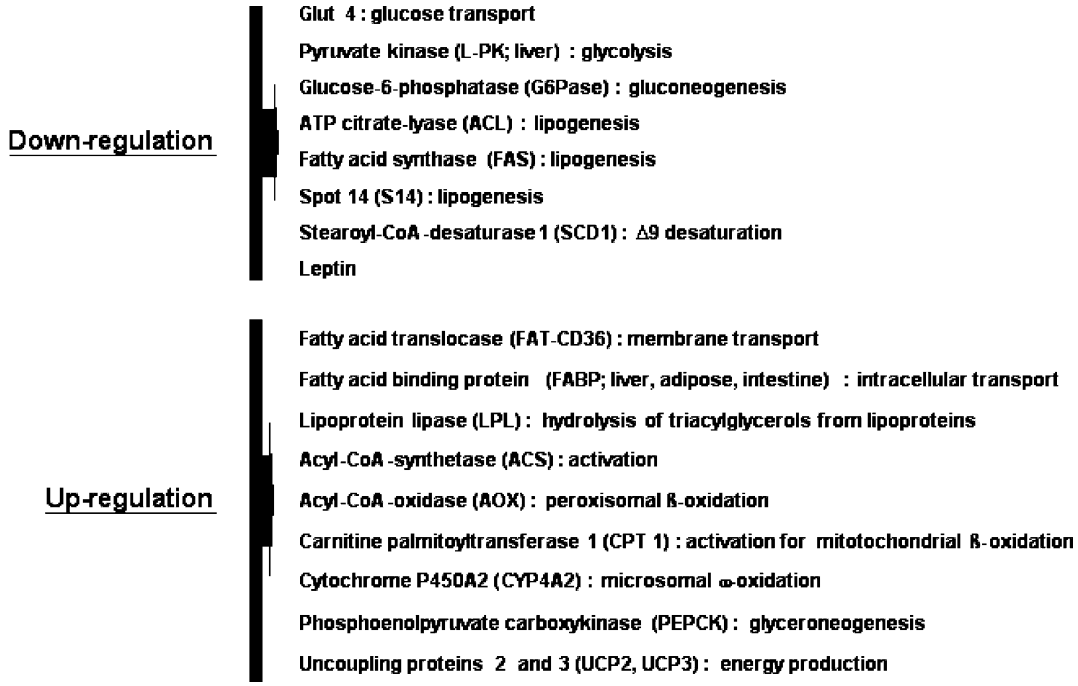

Fig. 1. Nonexhaustive list of FA-regulated genes and role of the corresponding proteins. The original reports describing the regulation by FAs of the genes mentioned in this figure can be found in references [56,16].

cells, FAs have various destinies. For instance they can be elongated, desaturated, β-oxidized for energy production, peroxidized, exchanged with phospholipids, participate in or interfere with eicosanoid synthesis. Hence, not only FAs *per se* but also products of FA metabolism can be the active molecules. Moreover, some selectivity of FA mobilization during lipolysis has been described and could account for variations in gene regulation (reviewed in [1]). The mechanism(s) by which FAs modulate gene expression still remains largely unresolved. The purpose of the present review is to address this important issue. It does not pretend to be exhaustive and focuses on some clearly demonstrated transcriptional mechanisms. It discusses the direct vs. indirect nature of FA action on genes and attempts to demonstrate that various routes can be taken, arguing against the common idea that peroxisome proliferator activated receptors (PPARs) are always involved in this process.

2. Generalities about gene regulation

Studies aimed at elucidating the mechanisms by which an effector regulates expression of a specific gene should always follow the same sequence of analyses. First, the transcriptional vs. post-transcriptional step of regulation should be determined (Fig. 2). In a first step, one should establish the cell or tissue culture system that maintains *in vitro* the physiology of the target tissue. Defining how the steady-state mRNA level changes in response to the effector is indicative of the physiological state of the cells but does not give a clue of the transcriptional nature of the effect. Run-on transcription experiments and transfection experiments with constructs containing the promoter of the target gene fused to a reporter gene, like for instance chloramphenicol acetyltransferase (CAT) of luciferase (Luc), should be carried out to address this question. Very often, inhibitors of gene transcription, like actinomycin D or 5,6-dichloro-1β-ribofuranosyl benzimidazole (DRB) are used instead. However, the risk of nonspecific and toxic effects linked to this class of compounds cannot be ruled out. Moreover, the effector can act indirectly (Fig. 2). In that case, a newly synthesized protein (protein X, Fig. 2) is required, the production of which would be prevented by actinomycin D or DRB acting by turning off transcription of the corresponding gene (gene *X*, Fig. 2). Such a result would suppose that FAs act by altering the synthesis of either a transcription factor or of some other protein with a rapid turnover and required for the effect. In such circumstance, the elucidation of the mechanism by which the effector act should also be shifted towards deciphering the nature of protein X (eventually a transcription factor—TF) and determining whether transcription of the gene encoding this protein is regulated by the effector. In contrast, the effect is defined as being direct if it is still achieved in the presence of protein synthesis inhibitors like cycloheximide, anisomycin or puromycin. Whether direct or not, post-transcriptional control can also occur. In that case, pulse-chase experiments or the use of transcription inhibitors could give some indications about the mechanism (Fig. 2). We chose to focus the present review on FA

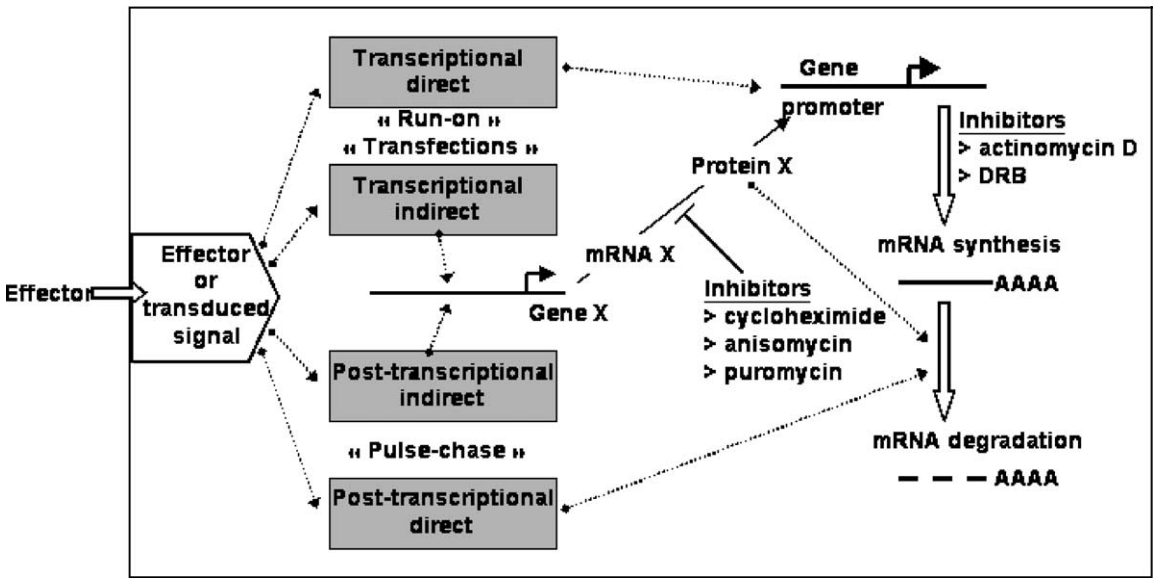

Fig. 2. Direct vs. indirect transcriptional and post-transcriptional regulation of gene expression. Among the steps leading from the regulated gene to the protein, an effector can modulate transcription rate and/or mRNA stability through direct or indirect routes. Run-on transcription and transfection experiments allow to determine whether transcription is affected but gives no information about the direct vs. indirect action of the effector. Protein synthesis inhibitors like cycloheximide, anisomycin or puromycin are employed to discriminate direct and indirect effects. Pulse-chase experiments or transcription inhibitors (actinomycin D, DRB ...) are used to get insights into post-transcriptional mechanisms of control.

regulation of genes for which the transcriptional nature of the effect has been clearly determined.

3. PPARs as FA-activated TF

PPARα, a member of the steroid/thyroid receptor superfamily, was first cloned over a decade ago by Issemann and Green [2]. It was originally shown that peroxisome proliferators (PP), xenobiotics and the hypolipidemic agents fibrates were pharmacological activators for this new receptor [2]. This discovery was rendered feasible by the establishment of a transactivation assay in which recipient cells (usually undifferentiated highly proliferative cells selected for their high transfection efficiency) are cotransfected with a PPAR expression vector and a reporter gene placed under the control of a transcription unit which contains a response element recognized and activated by the receptor. To date, three PPAR isoforms have been cloned: α, β/δ (NUC1 or FAAR) and γ, with tissue-selective expression, ligand-specific activation and ability to heterodimerize with retinoid X receptors (RXR) for which three isoforms: α, β, γ have also been isolated (reviewed in [3]). The PPAR/RXR heterodimer interact in target genes with PPAR response elements (PPREs). Consensus PPREs are direct repeat (DR) of AGGTCA separated by one nucleotide (DR1) with a 5′ extension of AACT for an increased specificity [3]. Using the above-mentioned transactivation assay, it was demonstrated that long-chain FAs (LCFAs), whether saturated or not, and certain prostaglandins (15-deoxy-$^{\Delta 12,14}$ prostaglandin J2) and other eicosanoids (8*S*-hydroyeicosatetraenoic acid; leukotriene B4) were PPAR activators [3–7]. These compounds were then proposed as natural ligands for all three PPARs. By analogy, the PPREs present in the promoter of regulated genes were defined as FA response elements. Among FAs, polyunsaturated FAs (PUFAs) were shown to be the most potent. Later on it was determined that these FAs and eicosanoids could indeed directly bind to PPARs [8–10]. More recently, the X-ray crystal structure of the ligand binding domain (LBD) of the three PPARs was determined ([11] and references herein). The LBD contains a cavity large enough to dock a variety of hydrophobic ligands with low affinity, in accordance with the promiscuous binding properties of the PPARs for eicosanoids and PUFAs. The later indeed occupies a volume of only about 30% of the pocket. To fit with these properties, the term of FA sensors has been proposed for PPARs. Since then, the established dogma is that regulated genes contain one or more PPREs in their promoter and respond to FAs *via* PPAR activation. However, recent data provided evidence that this scheme is probably not so simple.

4. Down regulation of gene transcription

4.1. The liver fatty acid synthase (FAS)

The mechanism by which FAs control transcription of the gene encoding the hepatic lipogenic enzyme FAS has made enormous progress lately although the first observation that feeding mice with linoleate (C18:2 N − 6) greatly depressed hepatic lipogenesis and FAS was made more

than 30 years ago [12]. This effect was shown to be restricted to PUFAs, since palmitate (C16:0) and oleate (C18:1 N − 9) were inefficient. Blake and Clarke [13] first showed that the reduction in hepatic FAS mRNA following the administration to rats of a PUFA-rich diet was caused primarily by the inhibition of gene transcription. N − 3 and N − 6 PUFAs were both equally efficient. Since prostanoid inhibitors failed to prevent PUFA action it was concluded that at least prostanoids are not involved [14]. In 1998, Worgall *et al*. [15] demonstrated that the monounsaturated FA oleate and PUFAs decreased the pool of mature sterol regulatory element-binding proteins 1 and 2 (SREBP 1 and SREBP 2), therefore down-regulating transcription of genes having SREBP response element (SRE) in their promoter. This was shown to be the case for the *FAS* and *S14* genes, leading to the proposal that dietary PUFAs would act on the so-called ''lipogenic genes'' by suppressing SREBP 1c expression (reviewed in [16]). The reason for which oleate decreases SREBP 1c expression but has no effect on lipogenic genes remains unclear. Very recently, Ou *et al*. [17] deciphered the mechanism by which unsaturated FAs inhibit transcription of *SREBP 1c*. In hepatoma cells, *SREBP 1c* gene expression is stimulated by agonists of liver X receptor (LXR), a nuclear hormone receptor. Unsaturated FAs competitively blocked activation of LXR by its ligand. Therefore, in that case, the mechanism of FA action on the lipogenic genes is indirect and PPARs are not involved.

5. Up regulation of gene transcription

5.1. The liver acyl-CoA oxidase (AOX) and carnitine palmitoyltransferase (CPT)

The AOX is a peroxisomal enzyme involved in FA oxidation. Berthou *et al*. [18] first showed that rats fed a high fat diet or primary hepatocytes exposed to the non-metabolized FA, α-bromopalmitate, presented a large increase in AOX mRNA. Using run-on assays they showed that α-bromopalmitate acted at the level of gene transcription. The promoter-regulatory region of the *AOX* gene contains a canonical PPRE which binds avidly the PPARα isoform and responds to FAs in transactivation assays in recipient cells of nonhepatic origin (reviewed in [3]). Owing to that, it was suggested that PPARα was the mediator of FA action on this gene. This assumption was further supported by data obtained with PPARα-deficient (PPARα −/−) mice [19]. These mice have lost the ability to respond to a PUFA-rich diet with an increase in liver AOX mRNA. Moreover, AOX mRNA can barely be induced by PUFAs in primary hepatocytes prepared from these PPARα −/− mice. Interestingly, they still respond to PUFAs by a repression of ''lipogenic gene'' expression, confirming that PPARα is not involved in the regulation of this class of genes.

The situation with the liver carnitine palmitoyltransferase 1 *(L-CPT 1)* gene is quite different from that of AOX. CPT 1 is the regulatory enzyme of mitochondrial β-oxidation. In primary hepatocytes from fetal rats L-CPT 1 mRNA is induced both by fibrates and LCFAs, whether saturated or not [20]. Activation and metabolism of LCFA are not required and the effect is indirect [20,21]. Both agents stimulate the transcription rate of the gene [20]. However, their mechanisms of action are divergent. First, insulin antagonizes the LCFA-induced transcription of *L-CPT 1* gene but not the PPARα-mediated clofibrate stimulation. Second, lipooxygenase inhibitors prevent clofibrate action whereas that of LCFAs is maintained. The final proof that FA action on the *L-CPT 1* gene is not PPAR-dependent was reported recently [21]. Using a series of deletions of *L-CPT 1*—Luc constructs transfected into Fao hepatoma cells, Louet *et al*. [21] demonstrated the existence of a LCFA-responsive region in the first intron of the gene, distinct from the well-known PPRE contained in the promoter. The response element remains to be located. Taken altogether these results show that LCFAs regulate transcription of the *L-CPT 1* gene through a PPARα-independent process although this gene is also responsive to fibrates.

These two examples of FA regulation, AOX and L-CPT 1, illustrate the fact that the same cells use different mechanisms to stimulate gene transcription in response to LCFAs. It should be mentioned in addition that LCFA induction of the *L-CPT 1* gene is not restricted to hepatocytes since it was also demonstrated in the pancreatic β-cell line INS-1 [22].

5.2. The fatty acid binding proteins (FABPs)

The FABPs are small cytosolic proteins that bind LCFAs with high affinity. These proteins play a crucial role in FA trafficking and in protection of cells against the adverse effects of FFAs [23,24]. Tissue-specific isoforms of FABPs are expressed. The mouse adipocyte FABP (ALBP) gene, named *aP2*, was cloned a couple of decades ago. It is selectively expressed in adipose tissue and is switched on during the process of adipocyte differentiation (adipogenesis) (reviewed in [25]). Spiegelman's laboratory demonstrated the existence in this gene of an adipocyte-specific enhancer located around −5 kilobase pairs (kbp) relative to the transcription initiation site [26]. Two elements in this enhancer have a DR1-type sequence and bind a PPARγ2/RXRα heterodimer [27]. In 1991, Amri *et al*. showed that FAs induced *ALBP/aP2* gene transcription in preadipose cells [28,29]. Saturated and unsaturated LCFAs are equipotent as is α-bromopalmitate, showing that metabolism is not required [30]. The stimulation of *aP2* gene expression is blocked by cycloheximide, indicating the occurrence of either an indirect mechanism or the involvement of other proteins, not directly working with the PPAR, required for a full activity of the promoter. The α-bromopalmitate has

adipogenic properties but is an inefficient PPARγ activator. In contrast, it was classified as a good PPARβ/δ activator (reviewed in [31]). The same is true for LCFAs. Grimaldi's laboratory demonstrated that genetically engineered fibroblasts overexpressing PPARβ/δ and exposed to LCFAs then to TZDs displayed an induction of PPARγ2 and of the *aP2* gene followed by terminal differentiation [32]. A transactivation-deficient form of PPARβ/δ which was mutated in the AF2 domain, severely blunted these effects [33]. Hence, PPARβ/δ appeared to play a central role in FA-controlled adipogenesis and *ALBP*/*aP2* induction.

Similarly, the liver FABP (L-FABP) is induced by LCFAs in hepatoma cells and in small intestine [34,35]. In both cases, an indirect mechanism appears to prevail. In small intestine, like in preadipocytes, PPARβ/δ seems to play the key role in LCFA induction [36].

5.3. The adipocyte phosphoenolpyruvate carboxykinase (PEPCK)

Results reporting FA regulation of gene transcription in mature adipocytes are scarce. In 1994 we demonstrated that oleate was a strong and rapid inducer of PEPCK mRNA in 3T3-F442A adipocytes [37]. This effect is direct and not due to a product of FA metabolism, affects the transcription rate of the gene and is restricted to unsaturated FAs, either monounsaturated or polyunsaturated [38]. More puzzling was the observation that among a series of reported potential target genes for direct FA regulation, PEPCK appeared quite unique in adipocytes [25,38,39]. Another puzzling observation was the cell-specific nature of FA action. Indeed, PEPCK mRNA is not affected by FAs in hepatoma cells whereas it is increased by other effectors, like cAMP or glucocorticoids [39]. This lack of effect is not due to a failure in FA action in these cells since the same treatment induces L-CPT 1 mRNA as expected.

These characteristics of FA control of *PEPCK* gene expression are quite in accordance with the role the enzyme plays in adipocytes. It was described more than 30 years ago as the key enzyme in glyceroneogenesis, a metabolic pathway which allows reesterification of FAs during lipolysis, therefore restraining FA output [40]. These results provided experimental evidence for the existence of a futile cycle in adipocytes, combining energy storage and release in fasting situations. Indeed, lipolysis and glyceroneogenesis are activated during fasting, a physiological situation during which glucose supply to the tissue is limited and glycolysis is reduced. This is consistent with our results showing that PEPCK mRNA induction by FAs in adipose tissue explants from rats and in 3T3-F442A adipocytes is repressed by glucose [37–39]. Although PEPCK is probably also glyceroneogenic in liver [41] the absence of hormone-sensitive lipolysis in hepatocytes argues for the lack of FA action on PEPCK in such cells.

Among a series of tested FAs, the very long-chain PUFA docosahexaenoic acid (DHA) is by far the most efficient

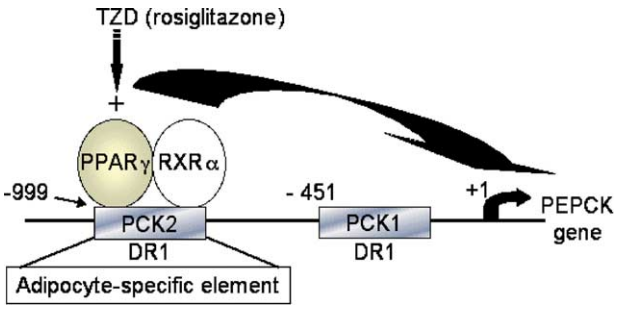

Fig. 3. A single element in the *PEPCK* gene mediates thiazolidinedione action specifically in adipocytes. The *PEPCK* gene promoter contains two DR1—type elements able to bind a PPARγ/RXRα heterodimer. Only one of these, PCK2, centered at −993 bp relative to the transcription start site, is the specific TZD-responsive element. PCK2 is also the adipocyte-specific element, the deletion of which prevents the expression of PEPCK in adipose tissue [43,57].

stimulator of PEPCK mRNA in adipocytes [38]. DHA is one of the best ligands for PPARs. The *PEPCK* gene promoter contains two distinct PPAR binding sites, PCK1 and PCK2 (Fig. 3) [42]. PCK2 is specifically bound by PPARγ from adipocyte nuclear extracts in gel shifts experiments [43–45] and is solely required for induction of the gene by the TZD class of compounds in transfection experiments (Fig. 3) [45]. These results plus the observation that neither PPAR activators nor DHA, affect *PEPCK* gene expression in hepatoma cells led us to hypothesize that PPARγ was the mediator of FA action on this gene [39,45]. To address this question, we transiently transfected 3T3-F442A adipocytes with the −2086 to +69 base pairs (bp) of the *PEPCK* gene promoter linked to the *CAT* gene (pPL1-*CAT*) and treated the transfected cells for 24 hr with either rosiglitazone (rosi), 1 μM or DHA, 320 μM in serum-free, glucose-deprived culture medium, in the presence of bovine serum albumin (BSA), 40 μM. We reported previously that, under these conditions, both agents nicely induce PEPCK mRNA in nontransfected cells [38,46]. We controlled that this was also the case with the transfected cells (not shown). Surprisingly, while rosi increased CAT activity about 3- to 4-fold, as expected, DHA had no effect (Fig. 4). We tested several additional conditions, varying DHA concentration in the presence of BSA or of 10% serum, changing the length of treatment (15–48 hr) or the period of DHA addition, i.e. either immediately following transfection or after a 24-hr lag; all this unsuccessfully.

The PPAR concentration could be limited in transfected adipocytes. To preclude such possibility, we transiently transfected 3T3-F442A adipocytes with pPL1 in the presence of varying concentrations of RXRα and either PPARα, PPARβ/δ or PPARγ expression vectors (gifts from Dr. Paul A. Grimaldi, Centre de Biochimie). We then treated the cells with bezafibrate as PPARα and β/δ activator or with rosi, in parallel to DHA. All of the PPAR expression vectors increased basal CAT activity 2- to 3-fold, which was additionally stimulated by the respective

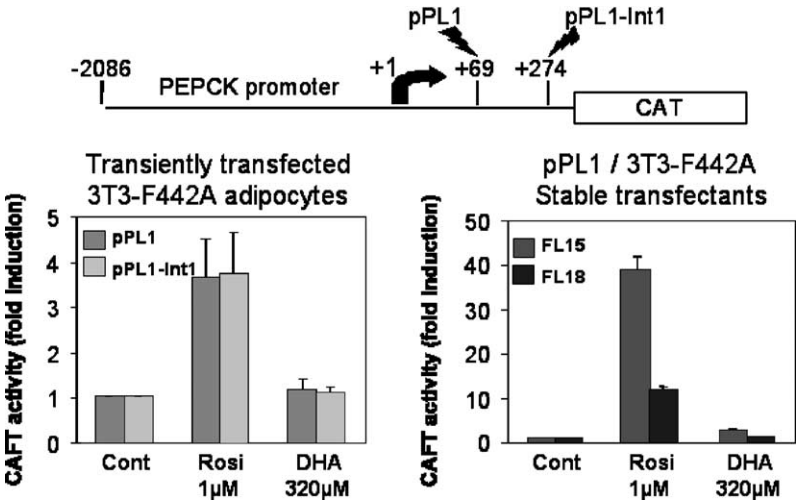

Fig. 4. Effect of rosi and of DHA on CAT activity in 3T3-F442A adipocytes transiently or stably transfected with PEPCK-CAT. Left panel; 10 days postconfluent 3T3-F442A adipocytes were transiently transfected by PEI-adenofection as described previously [45,58]. Two different CAT constructs were used. They contain the rat *PEPCK* gene promoter from −2086 to either +69 bp (pPL1) or to +274 bp (pPL1-Int1) relative to the transcription start site of the gene fused to the *CAT* gene. pPL1-Int1 encompasses the first exon and the first intron of the gene. Transfected cells were treated for 24 hr with either rosi, 1 μM or DHA, 320 μM in serum-free, glucose-deprived DMEM, in the presence of BSA, 40 μM, pyruvate, 1 μM and lactate, 0.1 μM. CAT activity was then determined as described [45]. Results represent the mean ± SEM from three independent experiments each performed in duplicate dishes. Right panel; the establishment of the FL15 and FL18 stable transfectants from 3T3-F442A cells was reported previously [59]. They contain the pPL1 construct stably integrated in their genome. Ten days postconfluent FL15 and FL18 adipocytes were treated by rosi or DHA as described above for the transient transfection experiments. Results of CAT activity in each transfectant are reported.

ligand, as expected (not shown). However, in none of these conditions had DHA any effect (not shown).

FAs might act on the *PEPCK* gene *via* the first intron, like they do on the *L-CPT 1* gene in hepatocytes. We made a construct containing −2086 to +274 bp of the rat *PEPCK* gene, therefore comprising the first exon and the first intron, fused to the *CAT* gene (pPL1-Int1) (Fig. 4). After transient transfection in 3T3-F442A adipocytes, pPL1-Int1 responded to rosi with the same magnitude as pPL1, but not to DHA (Fig. 4). We reasoned that FA effect on the *PEPCK* transgene construct might require its integration into chromatin. We made a series of stable 3T3-F442A transfectants with pPL1. In most of these transfectants, CAT activity is increased by rosi, although to various extents (not shown), while only two (FL15 and FL18) showed a weak response to DHA (2- to 3-fold), in contrast to the 12- to 40-fold induction by rosi in the same cells (Fig. 4).

Altogether these results converge to the conclusion that the mechanism of FA action on the *PEPCK* gene in adipocytes is different from that of TZD. We have shown that the later involves PPARγ and PCK2. Hence, although the exact mechanism of FA action on this gene still remains unknown, we can make the assertion that it is not as simple as requiring PPAR activation.

6. Overview of potential FA-sensor proteins

Besides PPARs, SREBP 1 and LXR, other transcription factors have been implied in FA effect on genes (Fig. 5). Among these, the orphan hepatic receptor hepatocyte nuclear factor 4 (HNF4) was shown to bind FA-CoA [47]. In transient transfection assays, oleate and PUFAs inhibit HNF4 transactivation potential on a construct containing the human apolipoprotein CIII gene promoter [47]. In contrast and unexpectedly, the saturated LCFA palmitate is stimulatory. This potential involvement of HNF4 in PUFA action has recently gained some physiological support. The expression of glucose-6 phosphatase, a key enzyme in gluconeogenesis, is repressed by PUFAs [48]. It was shown that the promoter-regulatory region of this gene contained HNF4 binding sites and that PUFAs specifically inhibited HNF4 binding to its cognate response elements, thereby suppressing its transactivation potency. In that case, PUFA-CoA thioester appeared to be the active moiety.

A similar situation was described with the thyroid hormone receptor (TR). The natural ligand for TR is triiodothyronine (T3). Studies indicated that FAs and their CoA esters could inhibit T3 binding to its receptor, thereby providing a potential mechanism for the FA repression of T3-dependent genes [49,50]. This observation is quite interesting since it was shown that TR and PPARs can compete for limiting amounts of their heterodimerization partner RXR [51]. The observation that PUFAs and more specifically DHA binds to all three RXR isoforms and transactivate RXR-responsive genes places also this transcription factor as a likely candidate for PUFA regulation [52]. Further work is required to ascertain these hypotheses.

The mechanism by which FAs repress glucose-induced *L-PK* gene transcription made some recent progress.

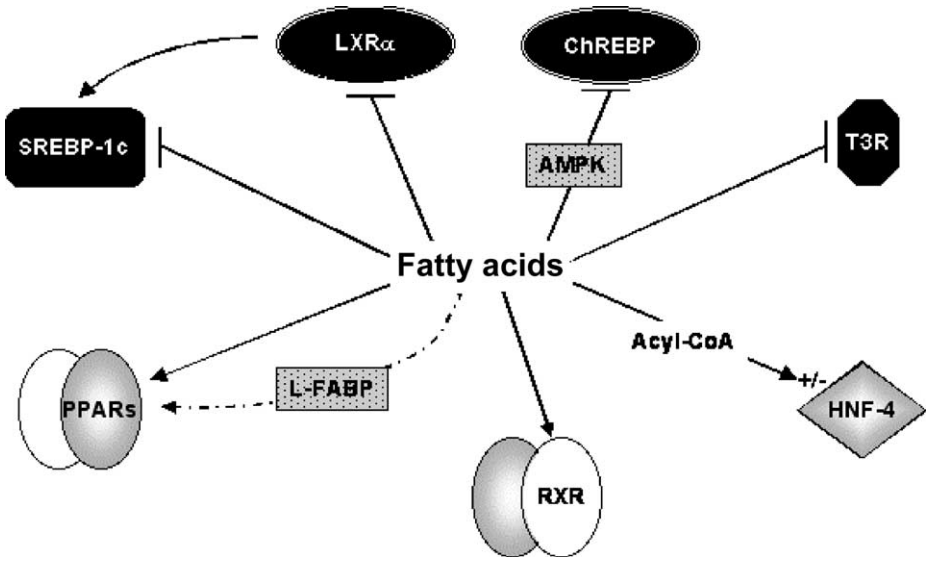

Fig. 5. Schematic overview of reported FA-sensor proteins. A series of transcription factors have been reported as being mediators of FA action on gene transcription. FAs can repress the transactivation potency of these factors. This appears to be the case for the SREBP 1c, LXR, TR or the ChREBP. In the later case, the AMPK might be involved. The PPARs are activated by LCFAs. The L-FABP was described as a PPAR-binding protein which could transport and deliver FAs to PPARα and γ. The situation with HNF4 is more complex. HNF4 transactivation property was reported to be repressed by PUFAs and activated by saturated FAs. In that case, PUFA-CoA ester is the active moiety. RXR, the dimerization partner for PPARs, TR and other nuclear receptors, was also shown as being a PUFA-binding protein.

Uyeda and colleagues cloned a new transcription factor, the carbohydrate response element binding protein (ChREBP), a mediator of the glucose stimulation of *L-PK* gene transcription [53]. They demonstrated that the ChREBP was the target for short-chain FA and LCFA repression of glucose-induced transcription and determined that the AMP-activated protein kinase (AMPK) was involved in this process [54].

Last but not least, the FABP itself could be involved in FA control of gene transcription. As already mentioned, FABPs bind FAs and facilitate their trafficking within cells. This protein could thus target and deliver FAs to their site of action. In support of this, Wolfrum *et al*. [55] showed recently that L-FABP and PPARα colocalize in the nucleus of primary hepatocytes and that both proteins interact directly in a ligand-dependent manner.

7. Conclusions

From several recent observations, it can be now inferred that the dogma stating that FA regulation of gene transcription is a simple PPAR-mediated process, should be revisited. In many instances the effects of PPAR ligands are disconnected from those of FAs. Indeed, there is no single mechanism for FA regulation of gene transcription. Depending upon the cell-specific context and the target gene, FAs can take very different routes to alter transcription. Several transcription factors different than PPARs are likely candidates and it can be assessed that new pathways of control will be soon discovered in this quickly evolving field.

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References

[1] Raclot T, Oudart H. Selectivity of fatty acids on lipid metabolism and gene expression. Proc Nutr Soc 1999;58:633–46.
[2] Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990;347:645–50.
[3] Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20:649–88.
[4] Gottlicher M, Widmark E, Li Q, Gustafsson JA. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. Proc Natl Acad Sci USA 1992;89:4653–7.
[5] 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 1993;90:2160–4.
[6] Yu K, Bayona W, Kallen CB, Harding HP, Ravera CP, Mcmahon G, Brown MS, Lazar MA. Differential activation of peroxisome prolif-

erator-activated receptors by eicosanoids. J Biol Chem 1995;270: 23975–83.
[7] Willson TM, Wahli W. Peroxisome proliferator-activated receptor agonists. Curr Opin Chem Biol 1997;1:235–41.
[8] Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci USA 1997;94:4312–7.
[9] Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci USA 1997;94:4318–23.
[10] Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol Endocrinol 1997;11: 779–91.
[11] Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JB, Oplinger JA, Kliewer SA, Gampe RT, McKee DD, Moore JT, Willson TM. Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proc Nat Acad Sci USA 2001;98:13919–24.
[12] Allmann DW, Gibson DW. Fatty acid synthesis during early linoleic acid deficiency in the mouse. J Lipid Res 1969;6:51–62.
[13] Blake WL, Clarke SD. Suppression of rat hepatic fatty acid synthase and S14 gene transcription by dietary polyunsaturated fat. J Nutr 1990;120:1727–9.
[14] Jump DB, Clarke SD, MacDougald O, Thelen AP. Polyunsaturated fatty acids inhibit S14 gene transcription in rat liver and cultured hepatocytes. Proc Natl Acad Sci USA 1993;90:8454–8.
[15] Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. J Biol Chem 1998;273:25537–40.
[16] Duplus E, Glorian M, Forest C. Fatty acid regulation of gene transcription. J Biol Chem 2000;275:30749–52.
[17] Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, Goldstein JL, Brown MS. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci USA 2001;98:6027–32.
[18] Berthou L, Saladin R, Yaqoob P, Branellec D, Calder P, Fruchart J-C, Denefle P, Auwerx J, Staels B. Regulation of rat liver apolipoprotein A-I apolipoprotein A-II and acyl-coenzyme A oxidase gene expression by fibrates and dietary fatty acids. Eur J Biochem 1995;232:179–87.
[19] Ren B, Thelen AP, Peters JM, Gonzalez FJ, Jump DB. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. J Biol Chem 1997;272:26827–32.
[20] Chatelain F, Kohl C, Esser V, McGarry JD, Girard J, Pegorier JP. Cyclic AMP and fatty acids increase carnitine palmitoyltransferase I gene transcription in cultured fetal rat hepatocytes. Eur J Biochem 1996;235:789–98.
[21] Louet JF, Chatelain F, Decaux JF, Park EA, Kohl C, Pineau T, Girard J, Pegorier JP. Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor alpha (PPARalpha)-independent pathway. Biochem J 2001;354:189–97.
[22] Assimacopoulos-Jeannet F, Thumelin S, Roche E, Esser V, McGarry JD, Prentki M. Fatty acids rapidly induce the carnitine palmitoyltransferase I gene in the pancreatic beta-cell line INS-1. J Biol Chem 1997;272:1659–64.
[23] Veerkamp JH, Vankuppevelt THMSM, Maatman RGHJ, Prinsen CFM. Structural and functional aspects of cytosolic fatty acid-binding proteins. Prostaglandin Leukot Essent Fatty Acids 1993;49:887–906.
[24] Bernlohr DA, Coe NR, Simpson MA, Hertzel AV. Regulation of gene expression in adipose cells by polyunsaturated fatty acids. Adv Exp Med Biol 1997;422:145–56.
[25] Forest C, Franckhauser S, Glorian M, Antras-Ferry J, Robin D, Robin P. Regulation of gene transcription by fatty acids, fibrates and prostaglandins: the phosphoenolpyruvate carboxykinase gene as a model. Prostaglandin Leukot Essent Fatty Acids 1997;57:47–56.
[26] Graves RA, Tontonoz P, Spiegelman BM. Analysis of a tissue-specific enhancer—ARF6 regulates adipogenic gene expression. Mol Cell Biol 1992;12:1202–8.
[27] Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Develop 1994;8:1224–34.
[28] Amri EZ, Bertrand B, Ailhaud G, Grimaldi PA. Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. J Lipid Res 1991;32:1449–56.
[29] Amri EZ, Ailhaud G, Grimaldi PA. Regulation of adipose cell differentiation. II. Kinetics of induction of the aP2 gene by fatty acids and modulation by dexamethasone. J Lipid Res 1991;32:1457–63.
[30] Grimaldi PA, Knobel SM, Whitesell RR, Abumrad NA. Induction of aP2 gene expression by nonmetabolized long-chain fatty acids. Proc Natl Acad Sci USA 1992;89:10930–4.
[31] Grimaldi PA. The roles of PPARs in adipocyte differentiation. Prog Lipid Res 2001;40:269–81.
[32] Bastie C, Holst D, Gaillard D, Jehl-Pietri C, Grimaldi PA. Expression of peroxisome proliferator-activated receptor PPARdelta promotes induction of PPARgamma and adipocyte differentiation in 3T3C2 fibroblasts. J Biol Chem 1999;274:21920–5.
[33] Bastie C, Luquet S, Holst D, Jehl-Pietri C, Grimaldi PA. Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation. J Biol Chem 2000;275:38768–73.
[34] Meunier-Durmort C, Poirier H, Niot I, Forest C, Besnard P. Up-regulation of the expression of the gene for liver fatty acid-binding protein by long-chain fatty acids. Biochem J 1996;319:483–7.
[35] Niot I, Poirier H, Besnard P. Regulation of gene expression by fatty acids: special reference to fatty acid-binding protein (FABP). Biochimie 1997;79:129–33.
[36] Poirier H, Niot I, Monnot MC, Braissant O, Meunier-Durmort C, Costet P, Pineau T, Wahli W, Willson TM, Besnard P. Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. Biochem J 2001;355:481–8.
[37] Antras-Ferry J, Lebigot G, Robin P, Robin D, Forest C. Stimulation of phosphoenolpyruvate carboxykinase gene expression by fatty acids. Biochem Biophys Res Commun 1994;203:385–91.
[38] Antras-Ferry J, Robin P, Robin D, Forest C. Fatty acids and fibrates are potent inducers of transcription of the phosphenolpyruvate carboxykinase gene in adipocytes. Eur J Biochem 1995;234:390–6.
[39] Duplus E, Glorian J, Tordjman J, Berge R, Forest C. Evidence for selective induction of phosphoenolpyruvate carboxykinase gene expression by unsaturated and nonmetabolized fatty acids in adipocytes. J Cell Biochem 2002;85:651–61.
[40] Reshef L, Hanson RW, Ballard FJ. A possible physiological role for glycerolneogenesis in rat adipose tissue. J Biol Chem 1970;245:5979–84.
[41] Kalhan SC, Mahajan S, Burkett E, Reshef L, Hanson RW. Glyceroneogenesis and the source of glycerol for hepatic triacylglycerol synthesis in humans. J Biol Chem 2001;276:12928–31.
[42] Tontonoz P, Hu ED, Devine JH, Beale EG, Spiegelman BM. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 1995;15:351–7.
[43] Devine JH, Eubank DW, Clouthier DE, Tontonoz P, Spiegelman BM, Hammer RE, Beale EG. Adipose expression of the phosphoenolpyruvate carboxykinase promoter requires peroxisome proliferator-activated receptor gamma and 9-cis-retinoic acid receptor binding to an adipocyte-specific enhancer in vivo. J Biol Chem 1999;274:13604–12.

[44] Eubank DW, Duplus E, Williams SC, Forest C, Beale EG. Peroxisome proliferator activated receptor γ (PPARγ) and chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) negatively regulate the phosphoenolpyruvate carboxykinase promoter via a common element. J Biol Chem 2001;276:30561–9.

[45] Glorian M, Duplus E, Beale EG, Scott DK, Granner DK, Forest C. A single element in the phosphoenolpyruvate carboxykinase gene mediates thiazolidinedione action specifically in adipocytes. Biochimie 2001;83:933–43.

[46] Glorian M, Franckhauser-Vogel S, Robin D, Robin P, Forest C. Glucocorticoids repress induction by thiazolidinediones, fibrates, and fatty acids of phosphoenolpyruvate carboxykinase gene expression in adipocytes. J Cell Biochem 1998;68:298–308.

[47] Hertz R, Magenheim J, Berman I, Bar-Tana J. Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. Nature 1998; 392:512–6.

[48] Rajas F, Gautier A, Bady I, Montano S, Mithieux G. Polyunsaturated fatty acyl-coenzyme A suppress the glucose-6 phosphatase promoter activity by modulating the DNA binding of hepatocyte nuclear factor 4 alpha. J Biol Chem 2002;25:25.

[49] Inoue A, Yamamoto N, Morisawa Y, Uchimoto T, Yukioka M, Morisawa S. Unesterified long-chain fatty acids inhibit thyroid hormone binding to the nuclear receptor. Solubilized receptor and the receptor in cultured cells. Eur J Biochem 1989;183:565–72.

[50] Yamamoto N, Li QL, Mita S, Morisawa S, Inoue A. Inhibition of thyroid hormone binding to the nuclear receptor by mobilization of free fatty acids. Horm Metab Res 2001;33:131–7.

[51] Hunter J, Kassam A, Winrow CJ, Rachubinski RA, Capone JP. Crosstalk between the thyroid hormone and peroxisome proliferator-activated receptors in regulating peroxisome proliferator-responsive genes. Mol Cell Endocrinol 1996;116:213–21.

[52] de Urquiza AM, Liu S, Sjoberg M, Zetterstrom RH, Griffiths W, Sjovall J, Perlmann T. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 2000;290:2140–.

[53] Yamashita H, Takenoshita M, Sakurai M, Bruick RK, Henzel WJ, Shillinglaw W, Arnot D, Uyeda K. A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. Proc Natl Acad Sci USA 2001;98:9116–21.

[54] Kawaguchi T, Osatomi K, Yamashita H, Kabashima T, Uyeda K. Mechanism for fatty acid sparing effect on glucose-induced transcription regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. J Biol Chem 2002;277:3829–35.

[55] Wolfrum C, Borrmann CM, Borchers T, Spener F. Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha- and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. Proc Natl Acad Sci USA 2001;98:2323–8.

[56] Jump DB, Clarke SD. Regulation of gene expression by dietary fat. Annu Rev Nutr 1999;19:63–90.

[57] Olswang Y, Cohen H, Papo O, Cassuto H, Croniger CM, Hakimi P, Tilghman SM, Hanson RW, Reshef L. A mutation in the peroxisome proliferator-activated receptor gamma—binding site in the gene for the cytosolic form of phosphoenolpyruvate carboxykinase reduces adipose tissue size and fat content in mice. Proc Natl Acad Sci USA 2002;99:625–30.

[58] Meunier-Durmort C, Grimal H, Sachs LM, Demeneix BA, Forest C. Adenovirus enhancement of polyethylenimine-mediated transfer of regulated genes in differentiated cells. Gene Ther 1997;4:808–14.

[59] Antras-Ferry J, Franckhauser S, Robin D, Robin P, Granner DK, Forest C. Expression of the phosphoenolpyruvate carboxykinase gene in 3T3-F442A adipose cells: effects of retinoic acid and differentiation. Biochem J 1994;302:943–8.