When precursors are also regulators

As well as being the precursors of the triacylglycerols deposited as fat in adipose tissue, long-chain fatty acids are one class of agents that induce the differentiation of preadipocytes to adipocytes.

Adipose triglycerol stored in fat cells (adipocytes) is the major energy reserve of higher animals and can be deposited in huge amounts in humans when caloric intake continually exceeds expenditure. Adipocytes presumably emerged during evolution because their fat-storing capacity allowed survival during periods of energy deprivation or famine. In modern (particularly western) societies, however, where survival depends on other factors, the availability of high-fat foods and a sedentary lifestyle have led to a high incidence of obesity and its accompanying health problems, notably cardiovascular disease and noninsulin-dependent diabetes.

Although the etiology of obesity is complex, there is compelling evidence for a substantial genetic component. This view was recently reinforced by the cloning of the mutant gene responsible for obesity in a strain of genetically obese mice [1]. The wild-type gene encodes a protein secreted by adipocytes that is believed to act on the hypothalamus as a negative feedback agent to suppress appetite, increase energy expenditure and maintain hormonal balance in rodents. The *obese* gene is highly conserved in the human genome, suggesting that dysregulation or mutation of the gene may also contribute to human obesity [1].

An increased capacity for fat storage results from the expansion of the adipocyte population by differentiation from preadipocytes. The availability of preadipocyte cell lines that can differentiate into adipocytes in culture has allowed detailed analyses of the agents capable of inducing preadipocyte differentiation, and has led to the discovery of putative 'master regulators', that is, key transcription factors, whose expression is sufficient to induce adipose conversion. To fulfil their energy storage function, adipocytes must either import or synthesize the long-chain fatty acids used to fabricate triacylglycerol for intracellular deposition. Recent studies suggest that long-chain fatty acids themselves may also act as one of the agents inducing preadipocyte differentiation by triggering the expression and/or activation of the master regulators, thereby promoting expansion of the adipocyte population and of fat storage.

External inducers are required in order to trigger the differentiation of preadipocytes into adipocytes (reviewed in [2]). Although insulin-like growth factor 1, glucocorticoids and cyclic AMP (or a cyclic AMP phosphodiesterase inhibitor) are well-established inducers, fatty acids and the peroxisome proliferators (agents that induce peroxisome formation) have only recently come onto the scene as activators of adipogenesis in preadipocytes (Fig. 1). Both fatty acids and peroxisome proliferators are amphipathic and have carboxyl groups, although their other structural features are dissimilar. Nevertheless, it is likely that fatty acids and peroxisome proliferators induce adipocyte gene expression and differentiation by a common mechanism. Neither fatty acids nor peroxisome proliferators stimulate full differentiation alone, but they act synergistically with other external inducers to activate the differentiation program.

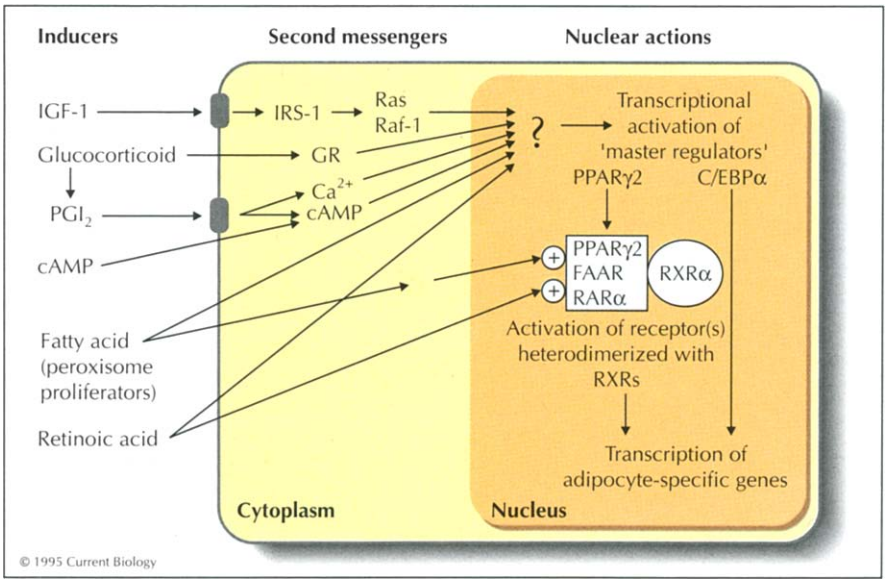

Fig. 1. The exogenous inducers, second messengers and pleiotropic transcription factors implicated in adipocyte differentiation. Physiological concentrations of retinoic acid have recently been shown to stimulate adipocyte differentiation through activation of retinoic acid receptor α (RARα) [16]. For a more detailed discussion of these inducers and second messenger systems, see [2].

How then do fatty acids and peroxisome proliferators activate adipogenesis? Adipocyte differentiation undoubtedly involves transcriptional activation of the genes that create and maintain the adipocyte phenotype. Our understanding of this process was recently advanced by the cloning of two members of the peroxisome proliferator-activated receptor (*PPAR*) gene family from mice, and by functional studies that implicate these genes as master regulators of the differentiation process. One of these genes encodes a nuclear hormone receptor, mPPARγ2, which binds to the adipocyte-specific enhancer of the gene for 422/aP2, a fatty-acid-binding protein expressed exclusively in adipocytes, in the form of a heterodimer with retinoid X receptor α (RXRα). Reports from Spiegelman's laboratory [3,4] reveal that mPPARγ2 is virtually identical to the previously cloned mPPARγ1 [5], except for an additional amino-terminal 30 amino acids. The structures of the PPARs are typical of other members of the steroid/thyroid hormone receptor superfamily. Both mPPARγ isoforms seem to be expressed predominately in white adipose tissue [3,6].

The mPPARγ isoforms are known to activate the transcription of genes for the adipocyte marker proteins 422/aP2 and phosphoenolpyruvate carboxykinase (PEPCK) through their binding to peroxisome proliferator response elements (PPREs) in the enhancers of these genes. As mPPARγ2 is specifically activated by both fatty acids and peroxisome proliferators, this also confers fatty-acid-inducibility on the *422/aP2* and *PEPCK* genes. But although peroxisome proliferators and fatty acids indirectly activate the PPARs, they do not seem to serve as ligands for these receptors. A physiological ligand(s) has not yet been identified, and this remains an important gap in our knowledge. The critical role of mPPARγ2 in adipose conversion has been shown by the forced expression of mPPARγ2 (and probably also of mPPARγ1) in fibroblasts. Treatment of fibroblasts with an activator of the receptor is sufficient to induce adipose conversion in these cells [4].

The other member of the PPAR family that regulates adipocyte gene expression is the fatty-acid-activated receptor (FAAR; also called mNUC1), which is the mouse homolog of the human NUC1. Grimaldi and colleagues [7] recently cloned the gene for this nuclear hormone receptor from a cDNA library derived from fatty-acid-induced mouse Ob1771 preadipocyte cells, by screening with a conserved *PPAR* nucleotide sequence. They observed that *FAAR* is expressed in a variety of lipogenic tissues (such as white adipose tissue, lung and intestine, but not liver), as well as certain non-lipogenic tissues (muscle, for example), and that, like other PPARs, FAAR binds to PPREs as a heterodimer with members of the RXR family. Like PPARγ2 [3,8], expression of FAAR in a fibroblastic cell line was found to confer fatty-acid-responsiveness on two endogenous adipocyte marker genes — those for the 422/aP2 fatty-acid-binding protein and a fatty acid transporter [7]. Thus, both PPARγ2 and FAAR can activate expression of adipocyte genes when transfected into fibroblast cell lines. Unlike mPPARγ2, however, FAAR seems to be more potently activated by fatty acids than by peroxisome proliferators, and it has not yet been demonstrated to be sufficient to induce adipose conversion [7].

The identification of two different PPAR-related transcription factors — the mPPARγ isoforms and FAAR — that activate expression of adipocyte markers raises the question of whether both function equally as modulators of preadipocyte differentiation, or whether one is of greater importance. The relative contributions of FAAR and mPPARγ2 might be evaluated by comparing the kinetics of their expression during the course of differentiation; this has provided equivocal results, however. Whereas Tontonoz *et al.* [3] found that mPPARγ2 was expressed shortly after induction of differentiation of 3T3-L1 and 3T3-F442A cells (within 1–2 days), Amri *et al.* [7] observed much later expression of mPPARγ2 during differentiation of Ob1771 cells. Similar discrepancies were observed in the timing of expression of *FAAR* during differentiation of these cell lines. These discrepancies underscore the difficulty of generalizing results obtained with different preadipocyte cell lines. Although these differences may be due, in part, to the efficacy of the agents used to induce differentiation, they are more likely to reflect differences in the stage of adipocyte development at which the different cell lines were arrested during cloning. All the preadipocyte cell lines achieve the same endpoint of terminal differentiation, but their entry points into the adipocyte differentiation program probably differ. Furthermore, different inducers and regulatory genes may be required to traverse the different stages of the developmental program.

The role of peroxisome proliferators in regulating lipid homeostasis is well established; they can induce differentiation of adipocytes as well as increasing lipid catabolism, depending upon the prevailing nutritional state. Indeed, a subclass of peroxisome proliferators, the fibrates, was initially developed as blood lipid-lowering agents for treatment of coronary heart disease. All of these agents increase both the size and number of hepatic peroxisomes, and induce peroxisomal β-oxidation of fatty acids by activating transcription of the genes encoding acyl-CoA oxidase and β-keto thiolase. The promoters of these genes contain PPREs to which PPARs bind, thus enabling transcriptional control indirectly by peroxisome proliferators and fatty acids.

The PPARs do not function alone to trigger adipocyte differentiation. Compelling evidence shows that expression of a basic region/leucine zipper transcription factor, C/EBPα (CCAAT/enhancer binding protein α), is also essential for the process [2]. Transcription of the C/EBPα gene is activated just before the coordinate expression of a group of adipocyte genes, the proximal promoters of which bind to, and are transactivated by, C/EBPα [9]. Definitive proof that this transcription factor is required for adipocyte differentiation was obtained by expression

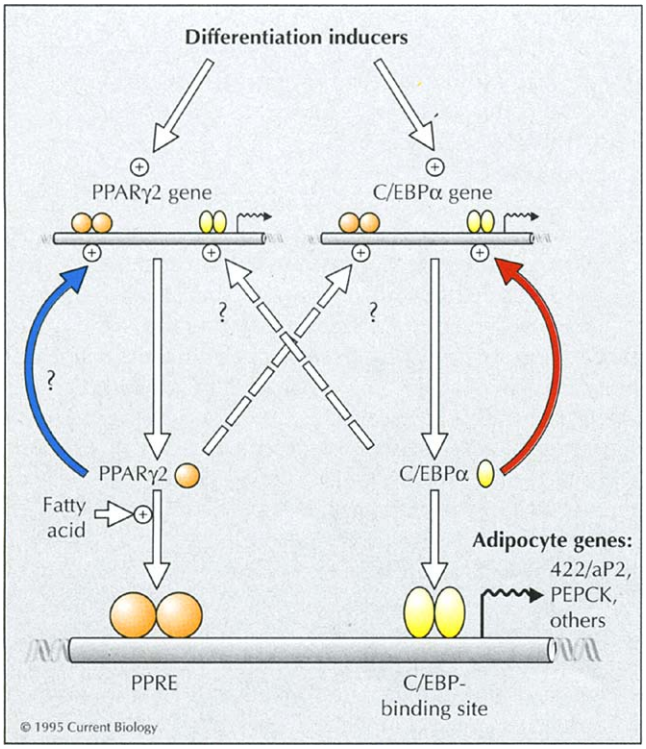

Fig. 2. Possible interactions between mPPARγ2, C/EBPα and their respective genes in the activation and maintenance of adipocyte differentiation. Transcriptional auto- and cross-activation by mPPARγ2 and C/EBPα are shown; question marks indicate inferred, but not proven regulation. The blue arrow refers to binding of PPAR and the red arrow to binding of C/EBPα to their respective *cis*-regulatory elements.

of its antisense RNA, which blocked adipocyte gene expression and the acquisition of the adipocyte phenotype [10]. Furthermore, premature induction of C/EBPα in 3T3-L1 preadipocytes using an inducible expression vector was sufficient to activate the differentiation program without external inducers [11]. Similarly, Freytag's group [12] showed that ectopic expression of C/EBPα was sufficient to induce adipose conversion in a variety of fibroblast and preadipocyte cell lines, several of which were not committed to the adipocyte lineage. These findings imply that expression of C/EBPα is both necessary and sufficient for adipocyte differentiation. C/EBPα null gene knockout' mice have a phenotype consistent with this hypothesis (G. Darlington, personal communication).

Tontonoz *et al.* [4], on the other hand, found that forced expression of both C/EBPα and mPPARγ2 in a fibroblast cell line was required in order to obtain maximal adipocyte differentiation. The fact that expression of either transcription factor alone caused little differentiation suggests that C/EBPα and mPPARγ2 act synergistically. Such a synergistic interaction would be consistent with the presence of functional binding sites for both C/EBPα and mPPARγ2 within the 5′ flanking regions of at least two genes induced during adipocyte differentiation, 422/aP2 [3] and *PEPCK* [8]. Perhaps mPPARγ2-mediated activation from the adipose-specific enhancers along with C/EBPα-mediated transactivation is sufficient to 'kick-start' their transcription. Maintenance of high levels of C/EBPα in terminally differentiated adipocytes is achieved through autoactivation of the C/EBPα promoter by C/EBPα [9,13]. As autoactivation is observed with many transcription factors involved in differentiation (such as MyoD), mPPARγ2 may also maintain its high level of expression through a positive feedback mechanism. The results of Tontonoz *et al.* [4] suggest that ectopic expression of C/EBPα in NIH 3T3 cells is sufficient to induce expression of mPPARγ2 and that ectopic expression of mPPARγ2 is sufficient to induce expression of C/EBPα. This suggests that, in addition to autoactivation, these transcription factors may cross-activate each other's expression, and thus enforce the maintenance of the fully differentiated state after the inducers of differentiation have disappeared. These concepts are illustrated in Figure 2.

Although the transcription factors C/EBPα and mPPARγ2 (and probably FAAR) are clearly involved in the transcriptional activation of genes that lead to development of the adipocyte phenotype, their roles in the fully differentiated adipocyte remain uncertain. Recent work with C/EBPα suggests that it may be involved in coordinating changes in adipocyte gene expression following differentiation in response to glucocorticoids and insulin [14,15]; mPPARγ2 and FAAR may also have roles in fully differentiated adipocytes by mediating the effects of fatty acids on adipocyte gene expression.

Substantial inroads have been made into our understanding of adipocyte differentiation and metabolism. The development of cell lines that differentiate into adipocytes in culture has facilitated detailed analyses of agents capable of inducing adipose conversion, and has led to the discovery of putative master regulators, such as C/EBPα and mPPARγ2 (and probably FAAR), whose expression is sufficient to induce adipose conversion. Future research should elucidate the mechanisms by which the exogenous inducing agents activate expression of these master regulators, and also how these regulators interact to activate the transcription of genes that create and maintain the adipocyte phenotype.

References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: **Positional cloning of the mouse *obese* gene and its human homologue.** *Nature* 1994, **372:**425–432.
2. MacDougald OA, Lane MD: **Transcriptional regulation of gene expression during adipocyte differentiation.** *Annu Rev Biochem* 1995, **64:**345–373.
3. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM: **mPPARγ2: tissue-specific regulator of an adipocyte enhancer.** *Genes Dev* 1994, **8:**1224–1234.
4. Tontonoz P, Hu E, Spiegelman BM: **Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor.** *Cell* 1994, **79:**1147–1156.
5. Zhu Y, Alvares K, Huang Q, Rao MS, Reddy JK: **Cloning of a new member of the peroxisomal proliferator-activated receptor gene family from mouse liver.** *J Biol Chem* 1993, **268:**26817–26820.
6. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA: **Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation.** *Endocrinology* 1994, **135:**798–800.
7. Amri E-Z, Bonino F, Ailhaud G, Abumrad NA, Grimaldi PA: **Cloning**

of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J Biol Chem* 1995, **270:**2367–2371.

8. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM: **PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene.** *Mol Cell Biol* 1995, **15:**351–357.
9. Christy RJ, Kaestner KH, Geiman DE, Lane MD: **CCAAT/enhancer binding protein gene promoter: Binding of nuclear factors during differentiation of 3T3-L1 preadipocytes.** *Proc Natl Acad Sci USA* 1991, **88:**2593–2597.
10. Lin F-T, Lane MD: **Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes.** *Genes Dev* 1992, **6:**533–544.
11. Lin F-T, Lane MD: **CCAAT/enhancer binding protein a is sufficient to initiate the 3T3-L1 adipocyte differentiation program.** *Proc Natl Acad Sci USA* 1994, **91:**8757–8761.
12. Freytag SO, Paielli DL, Gilbert JD: **Ectopic expression of the CCAAT/enhancer-binding protein a promotes the adipogenic program in a variety of mouse fibroblastic cells.** *Genes Dev* 1994, **8:** 1654–1663.
13. Lin F-T, MacDougald OA, Diehl AM, Lane MD: **A 30 kilodalton alternative translation product of the CCAAT/enhancer binding protein α message: transcriptional activator lacking antimitotic activity.** *Proc Natl Acad Sci USA* 1993, **90:**9606–9610.
14. MacDougald OA, Cornelius P, Lin F-T, Chen SS, Lane MD: **Glucocorticoids reciprocally regulate expression of the CCAAT/enhancer-binding protein α and δ genes in 3T3-L1 adipocytes and white adipose tissue.** *J Biol Chem* 1994, **269:** 19041–19047.
15. MacDougald OA, Cornelius P, Liu R, Lane MD: **Insulin regulates transcription of the CCAAT/enhancer binding protein (C/EBP) α, β, and δ genes in fully-differentiated 3T3-L1 adipocytes.** *J Biol Chem* 1995, **270:**647–654.
16. 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* 1994, **104:**201–211.

O.A. MacDougald and M.D. Lane, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.