Early adipocyte differentiation
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Introduction
An understanding of the mechanisms underlying adipose cell differentiation requires the identification of specific proteins that are preferentially expressed during the preadipose state, defined as the differentiation stage that precedes terminal differentiation characterized by the emergence of lipogenic enzymes and lipid accumulation. In contrast with terminal differentiation, the early stage of the adipose conversion process does not require adipogenic hormones [1]. It has been shown that this early stage in the differentiation of preadipocytes, giving rise to non-terminally differentiated cells, is coupled to growth arrest [2,3], but the characteristics of this state, the molecular events linked to growth arrest and the effectors regulating gene expression at that stage of the cell cycle remain poorly documented.

Growth arrest and expression of early markers
The necessity of growth arrest for adipose cell differentiation was clearly illustrated when Ob17 cells were transformed by the middle-T-only gene of polyoma virus. In the different clones obtained, an inverse relationship was observed between their potential to overproliferate at low serum and their potential to convert into adipose cells [4]. Moreover, exponentially growing cells were able, after growth arrest, to express early differentiation-specific markers. After a single- or double-thymidine block of actively growing cells, i.e. in the absence of intercellular contacts, the emergence of pOb24 mRNA and lipoprotein lipase (LPL) mRNAs in both Ob17 and 3T3-F442A cells was rapid. This transcriptional activation was confined to early markers, since late markers such as glycerol-3-phosphate dehydrogenase mRNA remained undetectable at that stage. The expression of pOb24 mRNA was studied in some detail, as it appeared to be
absent from both growing and growth-arrested 3T3-C2 cells, a clonal line showing a low frequency of adipose conversion [5]. Comparative studies of the expression of pOb24 and dihydrofolate reductase genes during the cell cycle have suggested that arrest at the G1/S boundary is critical for entry into the preadipose state [6]. In mouse adipose tissue, pOb24 mRNA was present at a high level in stromal–vascular cells (containing adipose precursor cells) and at a low level in mature adipocytes [7]. Thus pOb24 mRNA appears to be, both in vitro and in vivo, a unique marker of the preadipose state, i.e. of cell commitment during adipose differentiation. pOb24 mRNA was shown to be present as two transcripts of 3.7 and 6 kb generated from the same gene encoding a protein which shows strong similarities to the human α2 chain of type-VI collagen mRNA. Thus A2COL6/pOb24 encodes for an extracellular matrix protein [8]. The transient expression of A2COL6/pOb24 was recently observed at the protein level, in vitro and in vivo, by using immunohistochemical techniques (T. Kawada, C. Darimont, C. Deni and G. Ailhaud, unpublished work). Another interesting early marker, the transient expression of which is coupled to growth arrest, is clone 9. Cloning and sequencing of clone 9 have indicated that it encodes a protein of the pentaxin family (E. Amri and P. Grimaldi, unpublished work). This is rather surprising in view of the well-known production of pentaxins during inflammatory processes [9, 10].

Of the early events of adipose cell differentiation, the very rapid emergence of lipoprotein lipase [2] and a recently cloned and sequenced [11] fatty acid-activated receptor (FAAR), may help to provide insights into subsequent events (see below).

Relationship between the expression of early markers and terminal differentiation

The requirement of collagen synthesis for terminal differentiation was observed when commitment of adipoblasts to preadipocytes occurred in the presence of ethyl-3,4-dihydroxybenzoate, a specific inhibitor of collagen synthesis. Induction of early genes, i.e. A2COL6/pOb24 and LPL genes, was not prevented, in contrast with that of late genes, i.e. adipocyte lipid-binding protein (ALBP) and glycerol-3-phosphate dehydrogenase genes [12]. Similar observations were reported for the inhibition of muscle cell differentiation [13]. Clearly, preadipocytes should be able to respond at that stage to a limited combination of adipogenic hormones [1] and to fatty acids acting like hormones by means of FAAR [11]. This nuclear receptor is likely to be the mediator of fatty acid transcriptional effects in preadipocytes, and recent data have shown that transfection of 3T3-C2 fibroblasts with a FAAR expression vector conferred on the cells fatty acid inducibility of ALBP and a fatty acid transporter [11] as well as the ability to undergo terminal differentiation (P. Grimaldi and E. Amri, unpublished work). It is suggested that the emergence of LPL, which is able to provide the cells with fatty acids after growth arrest, represents a key event in the formation of preadipocytes. At that stage, preadipocytes rely on an exogenous supply of fatty acids for complex lipid biosynthesis, as endogenous synthesis from glucose is still undetectable, in contrast with adipocytes. Subsequently, fatty acids via FAAR and adipogenic hormones participate in the formation of adipocytes from preadipocytes [12]. In this hypothesis, preadipocytes, but not adipocytes, should be able to respond to adipogenic hormones acting within a critical ‘window of action’. This is indeed the case as, of the locally produced hormones, preadipocytes can only respond to an adipogenic hormone, namely prostacyclin [14], whereas adipocytes can only respond to an anti-lipolytic hormone, namely prostaglandin E2 [15].

Uncoupling protein in brown adipose tissue: molecular differentiation of the adipose tissues

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Introduction

The two forms of adipose tissue – 'brown' and 'white' – play very different roles in mammalian energetics. White adipose tissue (WAT) is the main long-term energy store in an animal, providing substrate in the form of fatty acids for utilization by other organs. In mammals, WAT is distributed in a number of locations throughout the body, both internal and subcutaneous. The vascularization of WAT is relatively sparse, as is the extent to which the tissue is innervated by the sympathetic nervous system. Up to 85% (by weight) of WAT may consist of triacylglycerol, and the white adipocyte is usually described as having a 'unilocular' arrangement of the lipid, i.e. there is a single fat droplet within the cell [1].

The main function of brown adipose tissue (BAT) is to generate heat, either for thermoregulation or in relation to the regulation of energy balance. Relative to WAT, BAT is restricted in terms of its anatomical localization, and it has an extensive vascularization and is densely innervated by sympathetic nerves [1]. The triacylglycerol content is generally rather lower in BAT (30–50% w/w) than in white fat. Traditionally, BAT is described as having a 'multilocular' disposition of fat droplets, i.e. a number of individual lipid droplets within each adipocyte. Table 1 summarizes the main differences between BAT and WAT; some authors have argued that there is a continuum of adipose tissues which are interconvertible, rather than there being two quite distinct forms [2,3].

Until recently, BAT and WAT have been largely differentiated on the basis of histological criteria, primarily the arrangement of the stored lipid (whether it is unilocular or multilocular), but this is now recognized as inappropriate. For example, in an obese animal, brown adipocytes appear unilocular rather than multilocular, whereas on fasting or during cold exposure white adipocytes may become multilocular through the loss of lipid [4–6]. This has led to a focus on molecular-based distinctions between brown and white fat, reflecting functional differences between the adipose tissues [7–9].

Overview of heat production in BAT

In addition to multiple lipid droplets, the major histological feature of active BAT is the presence of large numbers of mitochondria with a well-developed cristae structure [1]. There is a substantial oxidative capacity in BAT, but in contrast with mitochondria in other tissues the proton gradient that is generated during oxidation is not

Abbreviations used: BAT, brown adipose tissue; UCP, uncoupling protein; WAT, white adipose tissue.