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Review

Role of pathways for signal transducers and activators of transcription, and mitogen-activated protein kinase in adipocyte differentiation

J. Aubert, N. Belmonte and C. Dani*

Laboratoire de Biologie du Développement du Tissu Adipeux, Centre de Biochimie (UMR 6543 CNRS), F-06108 Nice (France), Fax + 33 4 92 07 64 04, e-mail: dani@hermes.unice.fr

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Abstract. Members of the signal transducer and activator of transcription (STAT) family and the mitogen-activated protein kinase (MAPK) cascade play a major role in the regulation of cell growth and differentiation. This review concentrates on the role played by these pathways in the development of adipose cells. STATs are activated by both positive and negative modulators of adipocyte differentiation leading to the hypothesis that the STAT pathway may function in adipogenesis. The role of the p42/p44 MAPK pathway in adipocyte differentiation has recently been the subject of contradictory reports. Several molecular mechanisms are proposed to explain the opposing effects of MAPK activation in the programme of adipose cell differentiation.

Key words. STAT; MAP kinase; adipocyte.

Introduction

Adipose cell differentiation is a multistep process characterised by a temporally defined sequence of events during which adipoblasts divide until confluence. Adipoblasts are fibroblast-like cells, and no specific genetic markers corresponding exclusively to this cell type have been identified. However, growth arrest leads to the emergence of early markers of differentiation, such as lipoprotein lipase and the α chain 2 of type VI collagen, and to the formation of preadipose cells that have not yet accumulated lipids. Upon hormonal addition, preadipose cells undergo terminal differentiation leading to the emergence of a large set of proteins involved in lipid accumulation and mobilisation. Key events of terminal differentiation have recently been identified by the characterisation of transcriptional factors playing a regulatory role in the differentiation process. The best-characterised factors identified as important in the development of mature adipocytes are members of the CCAAT/enhancer-binding protein (C/EBP) and peroxisome proliferative-activated receptor (PPAR) families. C/EBPs are nuclear proteins which have both leucine zipper and basic and acidic functional domains, whereas PPARs belong to the nuclear hormone receptor superfamily. Although both C/EBPs and PPARs are expressed by a range of cell types, PPARγ is highly expressed in adipocytes. Indeed, activation of PPARγ in preadipocytes has been shown to trigger the terminal differentiation process [1]. Generation by homologous recombination of C/EBPα-negative mice [2] or C/EBPβ/δ-negative mice [3] has established an essential role for these factors in the acquisition of adipocytes both in vitro and in vivo. It is now clear that these transcriptional factors play an overlapping role in the adipocyte differentiation process and the upregula-

* Corresponding author.

tion of C/EBPβ expression and C/EBPδ expression is to date the earliest known event occurring in preadipose cells as they traverse the differentiation process.

External inducers are required for the induction of terminal differentiation of preadipocytes into adipocytes, although the responsiveness of preadipose cells to external signals may vary according to the culture conditions tested and differences in the stage of development at which clonal lines have been established [4]. Glucocorticoid, insulin-like growth factor-1 growth hormone (GH), insulin, long-chain fatty acids, arachidonic acid and some of its metabolites, such as the prostacyclin PGI2, are inducers of differentiation. Mitogens and growth factors that stimulate preadipose cell growth, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), serve to inhibit adipocyte differentiation. An increasing body of evidence suggests that adipose tissue is an endocrine organ and that some factors secreted by the preadipocyte or the adipocyte may be involved in an autocrine/paracrine loop playing a role in the development of adipose cells [5].

Intracellular cAMP-elevating agents, such as prostacyclin via binding to its cell surface receptor (IP receptor), and activators of PPARs, such as fatty acids, promote the initiation of adipocyte differentiation [6–8]. The idea that signal transducer and activator of transcription STAT and mitogen-activated protein kinase (MAPK) signalling pathways could be involved in the regulation of adipocyte differentiation emerged recently following the observation that adipogenic and anti-adipogenic factors are potent activators of these two pathways.

STATs and adipocyte differentiation

STATs are a family of seven members (STAT1, 2, 3, 4, 5a, 5b and 6). STATs have been identified through the study of interferon induction of transcription. It is now clear that a wide variety of growth factors, hormones and cytokines activate the STAT pathway through a related family of cytokine receptors that activate members of the Janus kinase (JAK) family. Cytokine/receptor interactions promote JAK association, tyrosine phosphorylation of receptors and downstream signalling components. STATs are transcription factors located in the cytosol of unstimulated cells which, upon tyrosine phosphorylation, dimerise and translocate to the nucleus where they activate target gene transcription. STATs themselves are activated by a wide range of stimuli and their expression has been observed in all adult tissues tested and during embryogenesis in both mice and *Drosophila* [9]. STATs (STATs 1, 3 and 5) are expressed during differentiation of 3T3-L1 preadipocytes and in rat epididymal fat pad. The set of STATs activated in adipose cells differs from those activated in other cell types leading to the hypothesis that the STAT pathway may play a function in adipocytes [10, 11].

STATs can be activated by factors inducing opposite effects in adipocytes. Leptin is an adipocytokine involved in body weight homeostasis [12] which acts through the long form of the leptin receptor (OB-Rb) present in the hypothalamus and in certain peripheral organs including adipose tissue. Activation of OB-Rb in adipocytes induces an increase in the rate of lipolysis and it was proposed that this effect could be mediated via activation of STAT1 [13]. The subgroup of STATs activated by leptin seems to depend on the cellular context, as it was previously shown that only STAT3 is activated by leptin in the hypothalamus. In addition to activation of the STAT pathway, leptin can activate the p42/p44 MAPK pathway in transfected cells [14]. The entire complement of signalling pathways modulated by leptin in adipocytes is not fully known and the requirement of STAT1 for transducing the leptin signal in adipocytes has not yet been demonstrated.

Tumor necrosis factor (TNF)-α is a negative modulator of adipocyte differentiation. TNF-α has no effect on the activation of the JAK-STAT pathway in adipocytes [15]. However, the level of STAT1 protein is down regulated by TNF-α in mature adipocytes leading to the hypothesis that STAT1 may contribute to the effect of TNF-α in adipose cells [16]. The role of STAT1 in mediating TNF-α effects in adipose cells remains to be addressed experimentally.

STATs are also activated by factors playing a positive role in the development of the programme of adipocyte differentiation. Activation of STAT1 and STAT3 is induced by leukaemia inhibitory factor (LIF) in 3T3-L1 preadipose cells [11] and in 3T3-F442A and Ob1771 preadipose cells [J. Aubert and C. Dani, unpublished data]. Activation of STAT3 by LIF is required for self-renewal and maintenance of undifferentiated embryonic stem cells [17], differentiation of neuroepithelial precursors into astrocytes [18] and differentiation of myeloid leukaemic M1 cells [19]. However, the physiological consequences of STAT3 activation in preadipocytes are not yet determined. Identification of genes regulated by STATs in preadipose cells should be a first step to address this question. We have observed that LIF stimulates expression of the adipogenic transcription factors C/EBPβ and C/EBPδ and promotes terminal differentiation of Ob1771 preadipose cells in serum free-conditions [J. Aubert, N. Belmonte and C. Dani unpublished data]. The role of the STAT pathway in LIF-induced adipocyte differentiation remains to be analysed. GH promotes the differentiation of preadipocytes into adipocytes both in vitro and in vivo

[20–22]. The GH receptor belongs to the cytokine receptor superfamily and activates JAK2 tyrosine kinase and consequently STAT proteins [23]. Activation of JAK2 mediates GH effects in preadipose cells, as depletion of JAK2 using anti-sense oligodeoxynucleotides severely attenuates the ability of GH to promote adipocyte differentiation of 3T3-F442A preadipose cells [24]. Detailed studies have been performed in adipose cells showing that GH is a potent inducer of STAT5 activation [10]. Interestingly, a functional role of STAT5 in the inhibition of PPARα transcriptional activity induced by GH in COS cells has been reported [25]. This observation suggests that STAT and PPAR signalling pathways may cross-talk in preadipose cells and could be involved in the adipogenic effect of GH. However, the GH receptor can also activate other signalling pathways in preadipose cells, such as the protein kinase C (PKC) pathway [26] and the p42/p44 MAPK pathways [27]. Activation of STAT, MAPK and PKC pathways by GH depends on the cell type [28]. Cross-talk between these pathways is likely to exist in preadipose cells [29]. The role played by STAT and MAPK pathways in regulating the adipogenic effect of GH has recently been clarified. A serum-free medium has been developed in which GH priming of cells is required before terminal differentiation of 3T3-F442A preadipocytes. In this system, activation of p42/p44 MAPKs is not essential for GH priming but is necessary for terminal differentiation. In contrast, depletion of STAT5a and STAT5b completely abolishes the ability of GH to promote adipocyte differentiation [24]. Previously, GH-receptor deficient mice or STAT5a- and STAT5b-deficient mice were generated by homologous recombination and fat deposition was reduced [30]. Together, these results point to a role for the STAT pathway activated by GH in the development of adipose cells.

The p42/p44 MAPK pathway and adipocyte differentiation

MAPKs are a group of serine/threonine-specific protein kinases which are activated by a wide spectrum of extracellular stimuli. Activation of MAPK family members is achieved through kinase cascades that serve to connect cell surface receptors to specific transcription factors and other regulatory proteins [31]. The activation of p42/p44 MAPK plays a crucial role in cell growth and differentiation [32, 33]. Reports in the literature concerning the role of the MAPK pathways in adipocyte differentiation have been contradictory. p38 MAPK, a stress-activated MAPK, was recently reported to promote terminal differentiation of 3T3-L1 preadipose cells, in part through its effect on the activity of the adipogenic transcriptional factor C/EBPβ [34]. Equally, it has been suggested that p38 MAPK mediates the lipolytic effect of β-adrenergic agonists in mature adipose cells [35]. Sale et al. [36] have shown that depletion of p42/p44 MAPK using an anti-sense oligonucleotide strategy blocked the ability of 3T3-L1 preadipocytes to undergo differentiation in response to insulin. More recently, activation of p42/p44 MAPK was reported as required for terminal differentiation of 3T3-F442A preadipose cells induced by the combination of EGF, triiodothyronine and insulin in serum-free conditions [24]. In agreement with these results, we have observed that addition of PD98059, a specific inhibitor of the MAPK kinases MEK-1 and -2, inhibits LIF-induced adipocyte differentiation in serum-free conditions [unpublished data]. In contrast, Font de Mora et al. [37] have reported that inhibition of p42/p44 MAPK activation by PD98059 has no inhibitory effect on differentiation of 3T3-L1 preadipose cells in serum-supplemented medium. Moreover, adipocyte differentiation is inhibited in transfected 3T3-L1 cells constitutively expressing active MAPK. Several mechanisms could be proposed to explain these opposing effects of MAPK activation on adipogenesis. It has been shown that p42/p44 MAPKs are only activated during the early stages of adipocyte differentiation [34]. Therefore, the inhibitory effect of constitutive p42/p44 MAPK activation upon adipocyte differentiation could be stage dependent. Activation of this pathway in the inappropriate stage could render 3T3-L1 transfectants unable to undergo terminal differentiation.

An alternative mechanism favouring either positive or negative modulation of adipocyte differentiation by the p42/p44 MAPK pathway is the extent and the duration of p42/p44 MAPK activation. In CCL39 fibroblasts, the mitogenic activity of growth factors is only associated with a prolonged stimulation of MAPKs [38]. In PC12, EGF triggered cell proliferation or differentiation depending on the duration and extent of p42/p44 MAPK activation [39, 40]. Therefore, it is possible that constitutive activation of the MAPK pathway in 3T3-L1 transfectants promotes cell overproliferation, which is known to antagonise adipocyte differentiation [41]. Interestingly, the phosphorylation and activation of PPARγ by p42/p44 MAPK, which should trigger terminal adipocyte differentiation, has been associated with two opposing functional outcomes: on the one hand, phosphorylation of PPARγ by mitogen activated MAPKs, leads to the inhibition of adipogenesis, and direct phosphorylation of PPARγ following activation of MAPK by anti-adipogenic factors has been shown [42, 43]. On the other hand, activation of MAPKs by an adipogenic factor, i.e. insulin, enhanced adipogenesis and increased phosphorylation of PPARγ, although direct phosphorylation of PPARγ by MAPK in this case

remains to be demonstrated [44]. An activation of the p42/p44 MAPK pathway by different stimuli leading to opposite effects (proliferation or differentiation) in the case of preadipose cells has been previously reported for PC12 cells which can be induced to either proliferate or differentiate in response to a temporally distinct activation of p42/p44 MAPK [45]. Together, these results reflect the role played by the MAPK pathway in regulating the balance between cell growth and differentiation and indicate that the role of this signalling pathway should be studied under conditions where the additional effects of unidentified serum factors can be minimised. Detailed comparison of p42/p44 MAPK activation by adipogenic and anti-adipogenic factors in preadipose cells, including the extent and duration of the activation as well as nuclear translocation events [46], in association with PPARγ stimulation should bring further insights into the regulatory role played by the MAPK pathway in adipogenesis. The generation of mice mutant for p42 and/or p44 MAPK has not yet been reported but should shed new light on the role of the MAPK pathway in the development of adipose tissue.

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1 Hwang C. S., Loftus T. M., Mandrup S. and Lane D. M. (1997) Adipocyte differentiation and leptin expression. Annu. Rev. Cell. Dev. Biol. **13:** 231–259
2 Wang N. D., Finegold M. J., Bradley A., Ou C. N., Abdelsayed S. V., Wilde M. D. et al. (1995) Impaired energy homeostasis in C/EBP alpha knockout mice. Science **269:** 1108–1112
3 Tanaka T., Yoshida N., Kishimoto T. and Akira S. (1997) Defective adipocyte differentiation in mice lacking the C/EBPβ and/or C/EBPδ gene. EMBO J. **16:** 7432–7443
4 Smas C. M. and Sul H. S. (1995) Control of adipocyte differentiation. Biochem. J. **309:** 697–710
5 Ailhaud G., Grimaldi P. and Négrel R. (1992) Cellular and molecular aspects of adipose tissue development. Annu. Rev. Nutr. **12:** 207–233
6 Tontonoz P., Hu E. and Spiegelman B. M. (1994) Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell **79:** 1147–1156
7 Vassaux G., Gaillard D., Ailhaud G. and Négrel R. (1992) Prostacyclin is a specific effector of adipose cell differentiation: its dual role as a cAMP- and Ca^{2+}-elevating agent. J. Biol. Chem. **267:** 11092–11097
8 Amri E. Z., Ailhaud G. and Grimaldi P. A. (1994) Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells. J. Lipid Res. **35:** 930–937
9 Ihle J. (1996) STATs: signal transducers and activators of transcription. Cell **84:** 331–334
10 Han Y., Leaman D. W., Walting D., Rogers N. C., Groner B., Kerr I. M. et al. (1996) Participation of JAK and STAT proteins in growth hormone-induced signaling. J. Biol. Chem. **271:** 5947–5952
11 Stephens J. M., Lumpkin S. J. and Fishman J. B. (1998) Activation of signal transducers and activators of transcription 1 and 3 by leukaemia inhibitory factor, oncostatin-M, and interferon-gamma in adipocytes. J. Biol. Chem. **273:** 31408–31416
12 Maffei M., Fei H., Lee G. H., Dani C., Leroy P., Zhang Y. et al. (1995) Increased expression in adipocytes of mRNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc. Natl. Acad. Sci. USA **92:** 6957–6960
13 Siegrist-Kaiser C. A., Pauli V., Juge-Aubry C. E., Boss O., Pernin A., Chin W. W. et al. (1997) Direct effects of leptin on brown and white adipose tissue. J. Clin. Invest. **11:** 2858–2864
14 Nakashima N., Narazaki M. and Taga T. (1997) Overlapping and distinct signals through leptin receptor (OB-R) and a closely related cytokine signal inducer, gp130. FEBS Lett. **401:** 49–52
15 Jain R. G., Meredith M. J. and Pekala P. H. (1998) Tumor necrosis factor-alpha mediated activation of signal transduction cascades and transcription factors in 3T3-L1 adipocytes. Adv. Enzyme Regul. **38:** 333–347
16 Stephens J. M., Morrison R. F. and Pilch P. F. (1996) The expression and regulation of STATs during 3T3-L1 adipocyte differentiation. J. Biol. Chem. **271:** 10441–10444
17 Niwa H., Burton T., Chambers I. and Smith A. (1998) Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev. **12:** 2048–2060
18 Bonni A., Sun Y., Nadal-Vicens M., Bhatt A., Frank D. A., Rozovsky I. et al. (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science **278:** 477–483
19 Minami M., Inoue M., Wei H., Takeda K., Matsumoto M., Kishimoto T. et al. (1996) STAT3 activation is a critical step in gp130-mediated terminal differentiation and growth arrest of a myeloid cell line. Proc. Natl. Acad. Sci. USA **93:** 3963–3966
20 Doglio A., Dani C., Fredrikson G., Grimaldi P. and Ailhaud G. (1987) Acute regulation of insulin-like growth factor-I gene expression by growth hormone during adipose cell differentiation. EMBO J. **20:** 4011–4016
21 Doglio A., Dani C., Grimaldi P. and Ailhaud G. (1986) Growth hormone regulation of the expression of differentiated-dependent genes in preadipocyte Ob17 cells. Biochem. J. **238:** 123–129
22 Flint D. J. and Gardner M. J. (1993) Influence of growth hormone deficiency on growth and body composition in rats: site-specific effects upon adipose tissue development. J. Endocrinol. **137:** 203–211
23 Carter-Su S., Schwartz J. and Smit L. S. (1996) Molecular mechanism of growth hormone action. Annu. Rev. Physiol. **58:** 187–207
24 Yarwood S. J., Sale E. M., Sale G. J., Houslay M. D., Kilgour E. and Anderson N. G. (1999) Growth hormone-dependent differentiation of 3T3-F442A preadipocytes requires Janus kinase/signal transducer and activator of transcription but not mitogen-activated protein kinase or p70 S6 kinase signaling. J. Biol. Chem. **274:** 8662–8668
25 Zhou Y. C. and Waxman D. J. (1999) Cross-talk between Janus kinase-signal transducer and activator of transcription (JAK-STAT) and peroxisome proliferator-activated receptor-alpha (PPAR-alpha) signaling pathways. J. Biol. Chem. **274:** 2672–2681
26 Doglio A., Dani C., Grimaldi P. and Ailhaud G. (1989) Growth hormone stimulates *c-fos* gene expression by means of protein kinase C without increasing inositol lipid turnover. Proc. Natl. Acad. Sci. USA **86:** 1148–1152
27 Anderson N. G. (1993) Simultaneous activation of p90rsk and p70s6k S6 kinases by growth hormone in 3T3-F442A preadipocytes. Biochem. Biophys. Res. Commun. **193:** 284–290

28 Carter-Su C., King A. P., Argetsinger L. S., Smith L. S., Vanderkuur J. and Campbell G. S. (1996) Signalling pathway of GH. Endocrinol. J. **43:** S65–S70
29 MacKenzie F., Fleming I., Houslay M. D., Anderson N. G. and Kilgour E. (1997) Growth hormone and phorbol esters require specific protein kinase C isoforms to activate mitogen-activated protein kinases in 3T3-F442A cells. Biochem. J. **324:** 159–165
30 Teglund S., McKay C., Schuetz E., van Deursen J., Stravopodis D., Brown M. et al. (1998) Stat5a and STAT5b proteins have essential and nonessential or redundant roles in cytokine responses. Cell **93:** 841–850
31 Robinson M. J. and Cobb M. H. (1997) Mitogen-activated protein kinase pathways. Curr. Opin. Cell Biol. **9:** 180–186
32 Pagès G., Lenormand P., L'Allemain G., Chambard J. C., Meloche S. and Pouysségur J. (1993) Mitogen-activated protein kinase $p42^{mapk}$ and $p44^{mapk}$ are required for fibroblast proliferation. Proc. Natl. Acad. Sci. USA **90:** 8319–8323
33 Cowley S., Paterson H., Kemp P. and Marshall C. J. (1994) Activation of MAP kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. Cell **77:** 841–852
34 Engelman J. A., Lisanti M. P. and Scherer P. E. (1998) Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. J. Biol. Chem. **273:** 32111–32120
35 Moule S. K. and Denton R. M. (1998) The activation of p38 MAPK by the β-adrenergic agonist isoproterenol in rat epididymal fat cells. FEBS Lett. **439:** 287–290
36 Sale E. M., Atkinson P. G. P. and Sale G. J. (1995) Requirement of MAP kinase for differentiation of fibroblasts to adipocytes, for insulin activation of p90 S6 kinase and for insulin or serum stimulation of DNA synthesis. EMBO J. **14:** 674–684
37 Font de Mora J., Porras A., Ahn A. and Santos E. (1997) Mitogen-activated protein kinase activation is not necessary for, but antagonizes, 3T3-L1 adipocyte differentiation. Mol. Cell Biol. **17:** 6068–6075
38 Meloche S., Seuwen K., Pagès G. and Pouyssegur J. (1992) Biphasic and synergistic activation of p44mapk (ERK1) by growth factors: correlation between late phase activation and mitogenicity. Mol. Endocrinol. **6:** 845–854
39 Traverse S., Seedorf K., Paterson H., Marshall C. J., Cohen P. and Ullrich A. (1994) EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor. Curr. Biol. **4:** 694–708
40 Dikic I., Schlessinger J. and Irit L. (1994) PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentiation. Curr. Biol. **4:** 702–708
41 Grimaldi P., Czerucka D., Rassoulzadegan M., Cuzin F. and Ailhaud G. (1984) ob17 cells transformed by the middle-T-only gene of polyoma virus differentiate in vitro and in vivo into adipose cells. Proc. Natl. Acad. Sci. USA **81:** 5440–5444
42 Hu E., Kim J. B., Sarraf P. and Spiegelman B. M. (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. Science **274:** 2100–2103
43 Reginato M. J., Krakow S. L., Bailey S. T. and Lazar M. A. (1998) Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor γ. J. Biol. Chem. **273:** 1855–1858
44 Zhang B., Berger J., Zhou G., Elbrecht A., Biswas S., White-Carrington S. et al. (1996) Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor γ. J. Biol. Chem. **271:** 31771–31774
45 Nguyen T. T., Scimeca J. C., Filloux C., Peraldi P., Carpentier J. L. and Van Obberghen E. (1993) Co-regulation of the mitogen-activated protein kinase, extracellular signal-regulated kinase 1, and the 90-kDa ribosomal S6 kinase in PC12 cells: distinct effects of the neurotrophic factor, nerve growth factor, and the mitogenic factor, epidermal growth factor. J. Biol. Chem. **268:** 9803–9810
46 Lenormand P., Brondello J. M., Brunet A. and Pouyssegur J. (1998) Growth factor-induced p42/p44 MAPK nuclear translocation and retention requires both MAPK activation and neosynthesis of nuclear anchoring proteins. J. Cell Biol. **142:** 625–633