

REVIEW ARTICLE

CCAAT/enhancer-binding proteins: structure, function and regulation

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CCAAT/enhancer binding proteins (C/EBPs) are a family of transcription factors that all contain a highly conserved, basic-leucine zipper domain at the C-terminus that is involved in dimerization and DNA binding. At least six members of the family have been isolated and characterized to date (C/EBP α –C/EBP ζ), with further diversity produced by the generation of different sized polypeptides, predominantly by differential use of translation initiation sites, and extensive protein–protein interactions both within the family and with other transcription factors. The function of the C/EBPs has recently been investigated by a number of approaches, including studies on mice that lack specific members, and has identified pivotal roles of the family in the control of cellular proliferation and differentiation, metabolism, inflammation and numerous other responses, particularly in

hepatocytes, adipocytes and haematopoietic cells. The expression of the C/EBPs is regulated at multiple levels during several physiological and pathophysiological conditions through the action of a range of factors, including hormones, mitogens, cytokines, nutrients and certain toxins. The mechanisms through which the C/EBP members are regulated during such conditions have also been the focus of several recent studies and have revealed an immense complexity with the potential existence of cell/tissue- and species-specific differences. This review deals with the structure, biological function and the regulation of the C/EBP family.

Key words: differentiation, inflammation, metabolism, proliferation, transcription factors.

INTRODUCTION

The dramatic change in gene expression that accompanies processes such as differentiation, proliferation and the execution of specialized cellular function is controlled mainly at the transcriptional level. Such control is achieved through specific transcription factors that interact with regulatory sequences present in the promoter and enhancer regions of target genes. Studies in the last two decades on the regulation of numerous such genes have led to the identification of key transcription factors that act as master regulators of many cellular responses. Transcription factors belonging to the CCAAT/enhancer-binding protein (C/EBP) family fall in this category, with many pathophysiological conditions associated with their defective function.

The first C/EBP protein was identified in the laboratory of Steve McKnight as a heat-stable factor in rat liver nuclei that was capable of interacting with the CCAAT box motif present in several cellular gene promoters and a ‘core homology’ sequence found in certain viral enhancers [1]. The C/EBP gene was cloned in 1988 [2], and detailed studies on it led to the discovery of the basic-leucine zipper (bZIP) class of DNA-binding and dimerization domain [2–5], which is now known to be present in a battery of other transcription factors [6]. By 1992, five other

members of the C/EBP family had been identified, all of which contain a conserved bZIP domain at the C-terminus [7–14]. The function of the C/EBP family has been investigated in detail over the last decade and has identified pivotal roles for the proteins in numerous cellular responses, including the control of cellular growth and differentiation, immune and inflammatory processes and various diseases. The expression of the C/EBPs has also been found to change markedly during a number of physiological and pathophysiological conditions through the action of extracellular signals. This review summarizes key recent findings in relation to the structure, function and regulation of the C/EBP family.

STRUCTURE, PROPERTIES AND EXPRESSION PROFILE

The genes for six C/EBP members have been cloned to date from several species, with many of them being characterized independently in different laboratories and given distinct names (e.g. [7–14]). However, in order to avoid confusion, a systematic nomenclature was proposed by Cao and co-workers [12] in which members are designated as C/EBP followed by a Greek letter indicating the chronological order of their discovery (C/EBP α –C/EBP ζ). Table 1 lists the C/EBP genes that have been cloned from different species, along with the alternative

Abbreviations used: AP, activator protein; APR, acute-phase response; ATF, activating transcription factor; BAT, brown adipose tissue; BTEB, basic transcription element-binding protein; bZIP, basic-leucine zipper; C/EBP, CCAAT/enhancer-binding protein; CRE, cAMP-responsive element; CREB, cAMP-response-element-binding protein; CUP, C/EBP undifferentiated protein; EGF, epidermal growth factor; ER, endoplasmic reticulum; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte–macrophage colony stimulating factor; GRP, glucose-regulated protein; IFN- γ , interferon- γ ; IL, interleukin; LAP, liver activating protein; LIP, liver inhibitory protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony stimulating factor; mim-1, myb-induced myeloid protein-1; MIP, macrophage inflammatory protein; mTOR, mammalian target of rapamycin; NF, nuclear factor; NGF, nerve growth factor; PDGF, platelet-derived growth factor; PEPCK, phosphoenolpyruvate carboxykinase; PI 3-kinase, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PPAR, peroxisome-proliferator-activated receptor; Rb, retinoblastoma; RSK, ribosomal protein S-6 kinase; Runx, Runt domain factor; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TLS, translocated in liposarcoma; TNF α , tumour necrosis factor- α ; TPA, tumour promoter activators; USF, upstream stimulating factor; uORF, upstream open reading frame; UVC, UV light band C; VIP, vasoactive intestinal peptide; WAT, white adipose tissue.

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Table 1 Nomenclature of C/EBP genes

Gene*	Alternative name	Source	References
C/EBP α	C/EBP, RxC/EBP-1	Rat, mouse, human, chicken, bovine, <i>Xenopus laevis</i> , <i>Rana catesbeiana</i> , fish	[2,12,13,17–20]
C/EBP β	NF-IL6, IL-6DBP, LAP, CRP2, NF-M, AGP/EBP, ApC/EBP	Rat, mouse, human, chicken, bovine, <i>Xenopus laevis</i> , <i>Aplysia</i> , fish	[7–10,12,13,20–24]
C/EBP γ	Ig/EBP-1	Rat, mouse, human, chicken, fish	[11,20]
C/EBP δ	NF-IL6 β , CRP3, CELF, RxC/EBP2	Rat, mouse, human, <i>Rana catesbeiana</i> , bovine, ovine, fish	[12,13,19,20,25–27]
C/EBP ϵ	CRP-1	Rat, mouse, human, ovine, fish	[13,24,28–31]
C/EBP ζ	CHOP-10, GADD153	Mouse, rat, human, hamster	[14,32,33]

* It has not yet been possible to classify a *Drosophila* and a novel myeloid-restricted zebrafish C/EBP protein as homologues of these genes [15,16].

nomenclature. The C/EBP α , $-\beta$, $-\delta$ and $-\gamma$ genes are intronless, whereas C/EBP ϵ and $-\zeta$ contain two and four exons respectively [7–14,28–33]. An optimal C/EBP binding site has been determined to be a dyad symmetrical repeat RTTGCGYAAAY, where R is A or G, and Y is C or T [34]. However, substantial variations are tolerated, and most sites contain a conserved half-site paired with a more divergent sequence that contains at least 2 bp of the consensus [34].

All C/EBP isoforms share substantial sequence identity (> 90%) in the C-terminal 55–65 amino acid residues, which contains the bZIP domain [2–14]. This domain consists of basic-amino-acid-rich DNA-binding region followed by a dimerization motif termed the 'leucine zipper' [2–14]. Figure 1 shows the structure of the C/EBP β bZIP region homodimer bound to DNA. The leucine zipper of the bZIP domain consists of a heptad repeat of four or five leucine residues that assume an α -helical configuration, with two such repeats being able to interdigitate in a parallel manner to form a coiled-coil structure [3,4,6]. Electrostatic interactions between amino acids along the dimerization interface determine the specificity of dimer formation [4,6,37]. Dimerization is a prerequisite for DNA binding, which is mediated by the basic region, which also assumes an α -helical structure [4,6]. The basic region is in constant register relative to the leucine zipper, with changes in the spacing between the two domains leading to a loss of binding activity [5]. The specificity of DNA binding is dictated by the sequence of amino acids within the basic region [38]. According to a model for DNA binding by bZIP proteins [4,6], the dimer forms an inverted Y-shaped structure in which each arm of the Y is made of the basic region, which binds to one half of a palindromic recognition sequence in the DNA major groove like a fork or a pair of scissors (Figure 1).

Because of the high conservation in the bZIP domain, the different C/EBP proteins are able to form heterodimers in all intrafamilial combinations and, with the exception of C/EBP ζ , interact with an identical recognition sequence, at least *in vitro* [7–15,21–22,29]. C/EBP ζ , on the other hand, contains two proline residues in the basic region that disrupts its α -helical structure [14]. As a result, C/EBP ζ can readily dimerize with other members of the family, owing to the presence of an intact leucine zipper, but such heterodimers cannot bind to a C/EBP recognition sequence in the promoter of target genes [14]. However, C/EBP ζ /C/EBP heterodimers can bind to a different DNA sequence [consensus PuPuPuTGCAAT(A/C)CCC, where Pu is a purine] in the promoter regions of a subset of genes under conditions of cellular stress, and activate gene transcription [39]. Thus, C/EBP ζ can act both as an inhibitor of C/EBP function and as a direct activator of other genes, depending on the cellular state.

In contrast with the bZIP domain, the N-termini of the C/EBP proteins are quite divergent (< 20% sequence identity), except for three short subregions that are conserved in most members [40–45]. These three subregions have been shown variously to represent the activation domains (Figure 2) that interact with components of the basal transcription apparatus and stimulate transcription [40–44]. The major exception is C/EBP γ , which lacks an activation domain and, therefore, represses gene transcription by forming inactive heterodimers with other members [45]. The N-termini of some C/EBP proteins also contain negative regulatory regions (Figure 2) whose precise function remains to be elucidated in detail [40,42–44,46,47].

Despite the existence of six genes, the number of C/EBP proteins that can be present in any given tissue/cell type may be considerably higher (Figure 2). First, different sized polypeptides can be produced for C/EBP α and $-\beta$ either by alternative use of translation initiation codons in the same mRNA molecule due to a leaky ribosome scanning mechanism, or regulated proteolysis [48–51], and for C/EBP ϵ via alternative use of promoters and differential splicing [30,52]. Thus C/EBP α mRNA can give rise to two polypeptides, 42 kDa and 30 kDa, with the latter having a lower activation potential [49,50]. C/EBP β mRNA can produce at least three isoforms, 38 kDa (LAP*), 35 kDa (LAP) and 20 kDa (LIP), with the LAP and the LIP forms being the major polypeptides produced in cells [48]. LAP contains both the activation and the bZIP domains, whereas only the latter is present in LIP (Figure 2) [48]. LIP can, therefore, act as a dominant-negative inhibitor of C/EBP function by forming non-functional heterodimers with the other members [48]. For C/EBP ϵ , at least four isoforms can be produced (32 kDa, 30 kDa, 27 kDa and 14 kDa), of which, the activation potential of the 30 kDa form is lower than the 32 kDa form, and the 14 kDa form lacks an intact transcriptional activation domain (Figure 2) [30,52]. Secondly, as stated above, the different members are capable of forming heterodimers in all intrafamilial combinations [7–15]. Because the *trans*-activation potential of the various members differs [8,25,40,53], such heterodimerization is likely to have a profound effect on the regulation of target genes. Thirdly, the C/EBPs can form protein–protein interactions with other bZIP and non-bZIP transcription factors [54–56].

The tissue/cell expression profile of the different C/EBP isoforms has been determined at the level of both mRNA and protein [7–14,17,28–30,57]. However, some discrepancies between different reports on the expression pattern exist, which may be due to species-specific differences and/or the use of different techniques. In addition, for some members the expression of the protein does not correlate with that for mRNA (e.g. [9,13]), thereby indicating the extensive use of tissue-specific post-transcriptional regulatory mechanisms. In general, C/EBP α is



Figure 1 Structure of the C/EBP β basic region/leucine zipper domain bound to DNA

The two α -helical basic regions (bottom) dimerize through the α -helical leucine zipper domain (top) to form an inverted Y-shaped structure. Each arm of the Y is formed by a single α helix, one from each monomer, which binds to one-half of a palindromic recognition sequence. See the text and [35,36] for more details. The structure was produced from the Protein Data Bank entry 1H8A using the Rasmol program.

expressed at high levels in the adipose tissue, liver, intestine, lung, adrenal gland, peripheral-blood mononuclear cells and placenta [12,13,17,57]. In the liver and in adipose tissue, the highest levels of mRNA are present in the terminally differentiated cells [12,13,57]. Constitutive expression of C/EBP β is particularly high in the liver, intestine, lung, adipose tissue, spleen, kidney and myelomonocytic cells [7–10,12,13,21,22], whereas that for C/EBP δ occurs in the adipose tissue, lung and intestine [12,13,25]. On the other hand, C/EBP γ and C/EBP ζ are expressed ubiquitously [11,14], and C/EBP ϵ mRNA and protein are restricted primarily to myeloid and lymphoid cells [28–30,52]. The expression profile of different members is also regulated during physiological and pathophysiological changes by a range of extracellular mediators, and this is described in more detail below in the relevant sections.

FUNCTION OF THE C/EBP FAMILY

The function of the C/EBP family has recently been investigated in detail using a number of approaches, including analysis of the promoter regions of target genes, overexpression or inhibition approaches in cell-culture-based model systems and studies on knockout mice. These have identified pivotal roles of the family in a number of processes, including differentiation, the inflammatory response, liver regeneration, metabolism and numerous other cellular responses. These aspects are addressed below in detail, with Table 2 listing examples of target genes for C/EBP action during some of these processes.

Cellular differentiation

Adipocytes

Three classes of transcription factors, peroxisome-proliferator-activated receptor- γ (PPAR γ), C/EBPs and adipocyte determination and differentiation factor-1/sterol regulatory element-binding protein-1c, have been implicated as key regulators of adipogenesis [65–67]. The roles of these factors in adipocyte differentiation have been the subject of several excellent recent reviews [65–67], and thus the importance of only the C/EBPs will be described briefly. The original observation that C/EBP α is expressed at high levels in terminally differentiated cells of the adipose tissue promoted numerous studies investigating the role of the family in adipogenesis. The pre-adipocyte 3T3-L1 and the related 3T3-F442A cell lines have been used extensively to study this process [65–67]. Differentiation of growth-arrested pre-adipocytes is initiated by the addition of adipogenic hormones, which includes a cAMP elevating agent, a glucocorticoid, a hormone that interacts with the insulin-like-growth-factor receptors, and fetal-calf serum [65–67]. This treatment causes the pre-adipocytes to synchronously re-enter the cell cycle and undergo approximately two rounds of mitosis (mitotic clonal expansion) [65–67]. Both C/EBP β and C/EBP δ mRNA are induced during this phase in response to cAMP-elevating agents and glucocorticoid respectively [12]. The pre-adipocytes then exit the cell cycle and begin to express C/EBP α , which is then followed by the induction of adipocyte-specific markers [65–67]. Because the proximal promoter of the mouse C/EBP α gene can be activated by all three C/EBP isoforms (α , β and δ), owing to the presence of a C/EBP recognition sequence [88], it has been postulated that C/EBP α expression is activated by the binding of C/EBP β and C/EBP δ , which are induced early in the differentiation programme [12,67,88]. These two C/EBP isoforms have also been implicated in the induction of PPAR γ expression [89]. In addition, both C/EBP α and PPAR γ are able to induce each other's expression via a positive-feedback loop, which then promotes and maintains the differentiated state [65,66].

The importance of the C/EBP family in adipocyte differentiation has been confirmed by a number of approaches. First, ectopic expression of C/EBP α and C/EBP β in 3T3-L1 cells is able to initiate the differentiation programme in the absence of adipogenic hormones, whereas overexpression of C/EBP δ accelerates the process triggered by these agents [90–92]. Secondly, expression of antisense C/EBP α RNA in 3T3-L1 cells blocks differentiation [93]. Thirdly, embryonic fibroblasts lacking both C/EBP β and C/EBP δ are unable to initiate the differentiation programme in response to hormonal stimulation [94]. Fourthly, C/EBP α -deficient mice, which die soon after birth due to hypoglycaemia (see below), have dramatically reduced lipid accumulation in the adipose tissue [61].

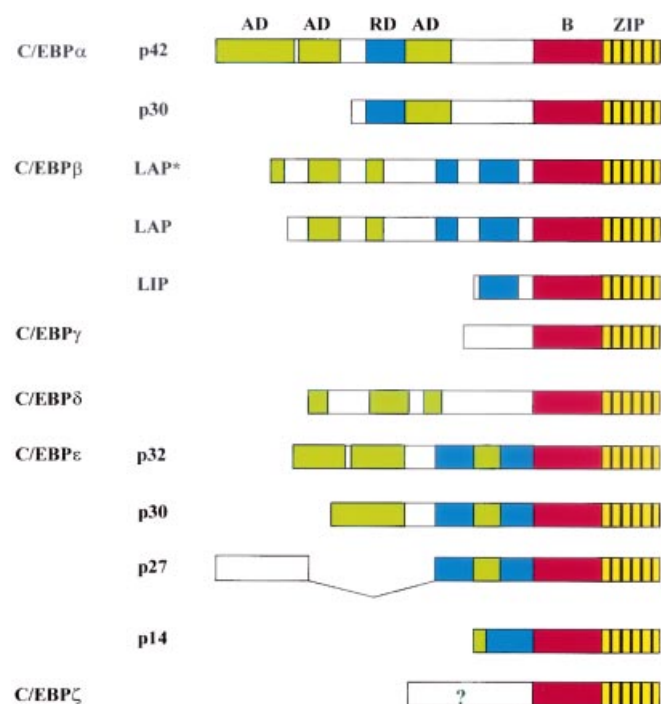


Figure 2 Schematic representation of the C/EBP family members

The leucine zipper is shown in yellow, with black vertical lines indicating the leucine residues, and the basic region is coloured red. The position of the activation domains (AD) and negative regulatory domains (RD) [40–44,47] are shown in green and blue respectively. ? indicates that the N-terminus of C/EBP ζ contains an activation domain, although its exact position remains to be determined [39]. The mechanism for the formation of the different C/EBP α , β and ϵ polypeptides is described in the text and [30,48–52].

Despite the advances detailed above, several aspects of C/EBP function during adipogenesis remain unresolved and require further detailed investigation. The first concerns the precise role of individual C/EBP members in the differentiation of white adipose tissue (WAT) versus brown adipose tissue (BAT) (see [66]). Some progress in addressing this aspect, however, has been made recently. For example, C/EBP α -deficient mice rescued from early death by re-expression of the gene in the liver show an absence of WAT in many depots, whereas their BAT has minimal biochemical alteration [95]. On the other hand, mice that lack either the β or the δ isoform have normal WAT, but their BAT show reduced accumulation of lipids and expression of uncoupling protein-1 [94]. Mice that lack both these C/EBP genes have a high rate of mortality in the perinatal period, but those that survive have drastically reduced BAT, with only slight decreases in WAT [94]. Another area for further investigation relates to the different pathways that operate in adipogenesis. For example, the observation that the WAT and BAT of both C/EBP β and C/EBP δ knockout mice express normal levels of PPAR γ and C/EBP α [94] implicates the existence of an alternative pathway for the activation of these two genes. Progress in the identification of the relative roles of C/EBP α and PPAR γ in adipogenesis has been made recently by the demonstration that whilst PPAR γ is able to promote adipogenesis in C/EBP α -deficient cells, the converse is not possible [96,97]. This suggests that C/EBP α and PPAR γ participate in a single pathway in adipogenesis, with PPAR γ being the most proximal effector of the process.

Myeloid cells

Binding sites for the C/EBPs are present in the promoter regions of numerous genes that are expressed in myeloid cells (Table 2). Indeed, four members of the family (α , β , δ and ϵ) are expressed in myeloid cells and show a unique expression profile in tissue-culture-based myeloid-cell differentiation systems [30,98–100]. For example, the expression of C/EBP α is relatively high in early myeloid progenitors and decreases during granulocytic differentiation [98]. On the other hand, the myeloid-restricted member, C/EBP ϵ , is preferentially expressed during granulocytic differentiation [30,99], whereas C/EBP β is up-regulated during macrophage differentiation [100]. In addition, ectopic expression of the C/EBPs has been shown to activate a number of target genes in myeloid cells [52,71,73,74]. However, the most compelling evidence for a crucial role of the C/EBPs in myeloid-cell differentiation and maturation has come from studies on knockout mice. Thus, C/EBP α -deficient mice fail to undergo myeloid differentiation beyond the myeloblast stage and, therefore, lack mature neutrophils [77]. This defect correlates with a lack of receptors for granulocyte colony stimulating factor (G-CSF) or interleukin-6 (IL-6), and expression of these receptors in C/EBP α -deficient progenitors restores granulopoiesis [77,78]. C/EBP ϵ -deficient mice, on the other hand, fail to produce atypical neutrophils, as indicated by morphological analysis, defective oxidative burst, delayed response to inflammatory challenge and impaired bacteriocidal responses [72,80]. In addition, these mice usually succumb to opportunistic infections by 3–5 months of age [72,80]. The phenotype of these mice is very similar to patients with a rare neutrophil-specific granulocyte deficiency [52]. Interestingly, two such patients have been shown to have defects in the C/EBP ϵ gene, which leads to the production of a non-functional protein [101,102]. More recently, macrophage functional maturation and cytokine production was found to be impaired in C/EBP ϵ -deficient mice [81]. Finally, the phenotype of C/EBP β -deficient mice indicates a potential role in the activation and/or differentiation of macrophages [71,103,104]. These mice are highly susceptible to infections by *Listeria monocytogenes*, *Candida albicans* and *Salmonella typhi*, which, in part, may be due to defects in macrophage activation and the escape of phagocytosed bacteria from the phagosome to the cytoplasm [71,103,104]. Other defects include impaired nitric oxide production by splenic macrophages, low levels of IL-12 (produced normally by activated macrophages) and impaired Th1 immune response [71,103,104].

Other cells

The importance of the C/EBP family in cellular differentiation is not restricted to adipocytes and myelomonocytic cells, but also extends to other cell types, including hepatocytes, mammary epithelial cells, ovarian luteal cells, keratinocytes, neuronal cells and intestinal epithelial cells [57,85–87,105–107]. Binding sites for the C/EBPs are present in the promoter regions of numerous genes in hepatocytes that are involved in the maintenance of normal function and responses to injury (Table 2). In addition, C/EBP α -deficient mice show profound derangement in liver structure with acinar formation, resembling proliferative or pseudoglandular hepatocellular carcinoma [82,108]. These results, therefore, implicate a pivotal role of the C/EBPs in the regulation of terminal hepatocyte differentiation and function. Analysis of C/EBP β knockout mice has shown that this isoform is required for the functional differentiation of mammary epithelial cells and the expression of milk protein genes [86,87]. In addition, the gene is required for the proper proliferation and

Table 2 Examples of target genes regulated by the C/EBP family

For brevity, further abbreviations have not been defined here; see the abbreviations footnote.

Tissue/cell type	Target gene	References
Hepatocytes	Albumin, α_1 -acid glycoprotein, bilirubin UDP-glucuronosyltransferase, C-reactive protein, complement C3, Factor IX, glucose-6-phosphatase, glycogen synthase, haptoglobin, haemopexin, ornithine-cycle-enzyme genes, PEPCK, serum amyloid A, tyrosine aminotransferase	[8–10,41,58–64]
Adipocytes	Fatty acid binding protein (aP2 or 422), insulin-responsive glucose transporter, leptin, obese, PPAR- γ , steroyl-CoA desaturase 1, uncoupling protein-1	[58,61,65–70]
Myeloid cells	CD14, cyclo-oxygenase-2, G-CSF, G-CSF receptor, GM-CSF receptor, IFN- γ , IL-1 β , IL-6, IL-8, IL-12 p40, inducible nitric oxide synthase, lactoferrin, lysozyme, MCP-1, M-CSF receptor, mim1, MIP-1 α , MIP-1 β , myeloperoxidase, neutrophil elastase, TNF- α	[21,52,58,71–81]
Lung	Clara-cell secretory protein, surfactant proteins	[82–84]
Keratinocytes	Keratin 1 and 10	[85]
Mammary gland	Milk protein genes	[86,87]

morphogenic responses during mammary-gland development [86,87]. Adult female C/EBP β -deficient mice also have been found to be sterile with the ovaries lacking the corpora lutea [105]. Further studies revealed that the isoform was essential for periovulatory granulosa cell differentiation in response to luteinizing hormone [105]. Finally, the C/EBPs have been implicated in the differentiation of neuronal cells, intestinal epithelial cells and keratinocytes on the basis of expression profile and/or presence of binding sites in the promoter regions of genes activated during the process, although the link needs to be investigated in detail [85,106,107].

Control of metabolism

Shortly after the discovery that C/EBP α was expressed at high levels in the liver, lung and the adipose tissue, McKnight and co-workers speculated that this isoform will be '... a central regulator of energy metabolism ...' [109]. This proposition has been found to be true not only for C/EBP α , but also for C/EBP β . As mentioned above, C/EBP α -deficient mice die soon after birth due to severe hypoglycaemia because of reduced expression of glycogen synthase, leading to undetectable levels of glycogen in the liver and decreased levels of three gluconeogenic enzymes [glucose-6-phosphatase, phosphoenolpyruvate carboxykinase (PEPCK) and tyrosine aminotransferase], which impairs the ability to synthesize glucose *de novo* [61]. These mice also have impaired expression of ornithine-cycle enzymes, leading to higher blood concentrations of ammonia compared with the wild-type counterpart [63]. Conditional knockout of the C/EBP α gene specifically in the liver also leads to major metabolic derangement due to reduced transcription of several genes, particularly those involved in glycogen synthesis, gluconeogenesis, and bilirubin detoxification [59]. As detailed above, the C/EBP α -deficient mice also have reduced lipid droplets in the adipose tissue [61].

Investigation of the exact role of C/EBP β in metabolism has been difficult because of at least two different phenotypes of knockout mice (A and B) [62]. Mice with the B-phenotype die soon after birth owing to hypoglycaemia, because of an inability to mobilize their hepatic glycogen and express PEPCK [110]. Mice with the A phenotype survive to adulthood, but display fasting hypoglycaemia, reduced blood lipids and impaired hepatic glucose production and adipose-tissue lipolysis in response to stimulation with hormones such as glucagon and adrenaline [110,111]. This has been attributed mainly to altered levels of hepatic cAMP production and the activity of protein kinase A [110,111].

Overall, these studies suggest that C/EBP α plays a more general role in energy metabolism, whereas C/EBP β is involved in fine-tuning responses to nutritional changes.

Inflammation

The activity and/or expression level of three C/EBP members (α , β and δ) is regulated by a number of inflammatory agents, including lipopolysaccharide (LPS) and a range of cytokines [7,8,25,53,60,71,112–115]. For example, the expression of C/EBP β and C/EBP δ mRNA is induced by inflammatory stimuli in a number of cell types, such as hepatocytes, macrophages, renal mesangial cells and astroglial cells, whereas that for C/EBP α is inhibited [7,8,25,53,60,71,112–115]. Indeed, C/EBP β was first identified on the basis of its ability to regulate gene transcription in response to IL-1 and IL-6 [7,8]. Subsequent studies have led to the identification of binding sites for the C/EBPs in the regulatory regions of a battery of genes that are involved in the inflammatory response, including those coding for cytokines and their receptors, acute-phase plasma proteins synthesized by hepatocytes, and components of signal transduction pathways (Table 2). Further support for an important role of the C/EBPs in the inflammatory response has been provided by studies on knockout mice. For example, C/EBP ϵ -deficient mice show delayed migration of neutrophils to an *in vivo* inflammatory challenge along with an impaired bactericidal response [80]. In addition, a number of genes that are involved in the immune and inflammatory responses are down-regulated in the macrophages of these mice [72,81]. C/EBP β -deficient mice show impaired expression of serum amyloid A and P proteins, α_1 -acid glycoprotein, complement C3 component and tumour necrosis factor- α (TNF α) [71,103,104]. However, not all the targets of C/EBP β action during an inflammatory response are affected in these mice [71]. It is likely that this may be due to functional compensation by other members that are expressed at normal levels. In addition, because the induction of both C/EBP β and C/EBP δ occurs relatively late following an inflammatory stimuli, it is possible that factors such as nuclear factor κ B (NF- κ B) and STATs (signal transducers and activators of transcription), whose activation during inflammation is much faster, and only transient, may be responsible for the primary induction of genes followed by the C/EBPs a few hours later [71].

Cellular proliferation

The initial observation that C/EBP α was expressed at high levels in terminally differentiated cells and down-regulated during proliferation (e.g. following partial hepatectomy) suggested an

antiproliferative role of this isoform [57]. Indeed, C/EBP α is a strong inhibitor of cell proliferation when overexpressed in cultured cells [57,116]. In addition, hepatocytes from newborn C/EBP α -deficient mice display increased proliferative activity, and similarly, hyperproliferation of alveolar type II cells is seen in the lungs of the animals [82]. More recently, mutations in the C/EBP α gene have been described in patients with acute myeloid leukaemia [117]. Although C/EBP α is a transcription factor, its ability to arrest growth does not require its DNA-binding activity, but is mediated via protein–protein interaction [118]. C/EBP α has been found to interact with several proteins that are involved in the control of cell-cycle progression, thereby indicating the existence of multiple pathways through which this isoform mediates growth arrest [118–123]. For example, the C/EBP α -mediated growth arrest is accompanied by increased expression of a cell-cycle inhibitor, p21, and the isoform has been shown to interact with, and thereby stabilize, the protein [119]. More recently, C/EBP α has been shown to interact with the cyclin-dependent kinases cdk2 and cdk4 and arrest cell proliferation by inhibiting their activity [122]. In the case of cdk4, it has been shown further that this interaction leads to a proteasome-dependent degradation of the protein [123].

C/EBP β , on the other hand, appears to play an important role in promoting proliferation, and its levels are increased in a number of tumours [124–127]. Partial hepatectomy is associated with increased expression of C/EBP β , and hepatocytes of mice deficient in this member do not proliferate normally during this process [124]. This abnormal regenerative response is associated with prolonged hypoglycaemia and altered expression of several genes that are important for hepatocyte gluconeogenesis and proliferation [124]. More recently, Zhu et al. [125] have shown that C/EBP β -deficient mice also are completely refractory to skin tumour development induced by a variety of carcinogens and carcinogenesis protocols. In v-Ha-ras transgenic mice, C/EBP β deficiency results in a significant reduction in tumorigenesis, thereby linking the proto-oncogene *ras* and C/EBP β [125]. In addition, oncogenic *ras* stimulates C/EBP β transactivation function, with mutation of an extracellular-signal-regulated-kinase-1/2 phosphorylation site (Thr¹⁸⁸), abolishing this effect [125]. Indeed, phosphorylation of C/EBP β is increasingly being found to be critical for its proliferative action [126,127]. For example, Buck et al. [126] have shown that an ribosomal protein S-6 kinase (RSK)-mediated phosphorylation of Ser¹⁰⁵ (rat) or Thr²¹⁷ (mouse) of C/EBP β is required for hepatocyte proliferation induced by transforming growth factor- α (TGF α). More recently, they have shown that the hepatotoxin carbon tetrachloride activates RSK, phosphorylates C/EBP β on Thr²¹⁷ and causes proliferation of hepatic stellate cells in normal mice [127]. In contrast, the toxin causes apoptosis of these cells in C/EBP β -deficient mice or transgenic animals expressing a non-phosphorylatable C/EBP β Ala²¹⁷ mutant [127]. They also showed that both [phospho-Thr²¹⁷]C/EBP β and the phosphorylation mimic [Glu²¹⁷]C/EBP β , but not [Ala²¹⁷]C/EBP β , are able to associate with procaspases 1 and 8, thereby providing a potential mechanism of action [127].

Other functions

A novel role for the C/EBPs in long-term synaptic plasticity underlying memory was identified by studies on the *Aplysia* C/EBP homologue, ApC/EBP [23]. Blocking the function of ApC/EBP by use of either antisense oligonucleotides or specific antibody leads to a selective inhibition of long-term facilitation [23]. Several lines of evidence suggest that such a role of C/EBP β

in memory is not only likely to be conserved in mammals, but also extend to other members of the family. First, C/EBP α , C/EBP β and C/EBP δ have been shown to be widely expressed in the mammalian nervous system [57,106,128–131]. Secondly, the expression and DNA binding activities of C/EBP β and C/EBP δ are enhanced in hippocampal neurons, which are involved in long-term memory, by two crucial inducers of the memory process, cAMP and Ca²⁺ [129]. Thirdly, using specific model systems, Taubenfeld et al. [130] have identified a key role of C/EBP β in mammalian long-term memory consolidation. Finally, C/EBP δ -deficient mice display a selectively enhanced contextual fear response, thereby implicating this isoform in learning and memory [131].

The expression of C/EBP ζ is induced by a number of agents that cause cellular stress, particularly in the endoplasmic reticulum (ER) [39,132]. More recently, C/EBP ζ has also been implicated in growth arrest and the control of apoptosis [133–135]. For example, C/EBP ζ -deficient mice are defective in the development of apoptosis in response to ER stress, such as that produced by agents that cause destruction of the pancreatic β -cells, and thereby diabetes [133–135]. In addition, most, if not all, human myxoid and round-cell liposarcomas are associated with chromosomal translocations that lead to gene fusions that specify for chimaeric oncoproteins consisting of an N-terminus from the *FUS* or *TLS* gene and a C-terminus from the C/EBP ζ gene [136,137]. In contrast with C/EBP ζ , the chimaeric protein fails to cause cell-growth arrest [138].

Finally, C/EBP γ -deficient mice show impaired natural killer-cell cytotoxic activity and interferon- γ (IFN- γ) production, thereby indicating a novel role of this isoform in the functional maturation of these cells [139].

REGULATION OF THE C/EBP FAMILY

The expression of the C/EBPs is regulated under several physiological and pathophysiological conditions through the actions of a number of factors, including cytokines, mitogens, hormones, nutrients and agents that cause cellular stress (Table 3). Understanding the mechanisms that are involved in the regulation of the C/EBPs during such conditions has been the focus of recent research in a number of laboratories, including our own. It is hoped that such studies will not only contribute to increasing our knowledge of the molecular basis of these processes but also lead to the identification of potentially novel targets for therapeutic intervention of diseases associated with perturbations in C/EBP gene expression. For instance, targeted suppression of C/EBP ζ expression in pancreatic β -cells has been recently proposed as a new therapeutic approach for preventing the onset of both type 1 and type 2 diabetes [134,135]. The C/EBPs have been found to be regulated at a number of levels, including gene transcription, translation, protein–protein interactions and phosphorylation-mediated changes in DNA-binding activity, activation potential and nuclear localization (see Table 3). In addition, the potential existence of tissue/cell- and species-specific differences in the mechanisms of regulation has been identified [57,58,113–115, 148,150,164–166]. For example, TNF α induces C/EBP β mRNA expression in astrocytes and renal mesangial cells, but modulates nuclear–cytoplasmic translocation in hepatocytes [113–115,148, 150]. The initial part of this section will describe the different control points that operate to regulate the C/EBP family and will be followed by a summary of the mechanisms that are involved in the global modulation of C/EBP expression in three important processes: adipocyte differentiation, acute-phase inflammatory response in hepatocytes and liver regeneration.

Transcriptional regulation

This represents the main point of control in the regulation of mRNA expression by factors shown in Table 3 [57,112]. The promoter regions of C/EBP isoforms α , β , δ , ϵ and ζ have been analysed. The role of the different sequence elements in the regulation of gene expression during adipogenesis, inflammation and hepatocyte proliferation is described below under global gene regulation, with other aspects addressed here.

C/EBP α

The proximal promoter region of the mouse C/EBP α gene was first characterized and shown to contain potential binding sites for C/EBP, Sp1, nuclear factor (NF)-1, NF-Y, upstream stimulating factor (USF), basic transcription element-binding protein (BTEB) and NF- κ B [88,167]. The promoter can be auto-activated in transfected cells by expression plasmids that specify for C/EBP α or C/EBP β , which act via a C/EBP recognition sequence [88,167]. The proximal promoter region of the rat C/EBP α gene shows almost complete sequence identity with the mouse counterpart and also can be auto-activated through the C/EBP-binding site [141]. On the other hand, the promoter regions of the human, chicken and *Xenopus* C/EBP α genes show only limited sequence identity with the mouse/rat promoter (e.g. 53% identity in the case of the human promoter) [164,166]. Nevertheless, both the *Xenopus* and the chicken promoters contain putative C/EBP recognition sequences and, in the case of the *Xenopus* promoter, we have shown that it can be auto-activated by C/EBP α or C/EBP β [164]. On the other hand, the human promoter lacks a C/EBP recognition sequence, owing to a single base substitution [166]. However, it can still be auto-activated, although only by C/EBP α , which acts indirectly via stimulation of the DNA-binding activity of USF, which interacts with a site present in the proximal promoter region [166]. These studies, therefore, show that the C/EBP α gene is auto-activated in a species-specific manner.

The regulatory sequences that are involved in the transcriptional activation of the C/EBP α gene by thyroid hormones and suppression by overexpression of the proto-oncogene *c-myc* have also been investigated [168–170]. The action of the thyroid hormone is mediated via a responsive element present at position –602 to –589 of the rat C/EBP α promoter [168]. On the other hand, the *c-Myc* protein inhibits C/EBP α gene transcription through interaction with the core promoter region, although a discrepancy exists on the precise sequence that is involved in *c-Myc* action [169,170].

C/EBP β

The promoters of the mouse, rat, chicken and *Xenopus laevis* C/EBP β genes have been characterized to date, and all four are subject to autoregulation [165,171–173]. The transcription factor CREB (cAMP-response-element-binding protein) also controls C/EBP β expression by interacting with two sites near the TATA box [174]. These cAMP-response element (CRE)-like sequences are also required for the IL-6-mediated induction of C/EBP β transcription during the acute-phase response (APR) through a novel pathway involving tethering of STAT-3 to a DNA-bound complex [175] (see below), and in conjunction with an Sp1 site for the activation of gene transcription during macrophage differentiation [176].

C/EBP δ

This isoform is expressed constitutively in osteoblasts, where it has been shown to activate the synthesis of insulin-like growth

factor I [177]. A binding site for the Runt domain factor (Runx) 2, which is essential for osteogenesis, is present in the 3' proximal region of the C/EBP δ gene promoter (–175/–147) [177]. The promoter activity is reduced drastically by mutation of this Runx-binding sequence or by co-transfection of a Runx2 anti-sense expression plasmid into osteoblasts [177]. The transcription of the C/EBP δ gene is also induced in growth-arrested mammary epithelial cells and in the involuting mammary gland through the activation of STAT-3, and its subsequent binding to a recognition sequence present in the promoter region [178,179]. This STAT site is also required for the IL-6-mediated induction of C/EBP δ transcription in hepatocytes during the APR [180,181] (see below). Although the activation of STAT-3 under such conditions is transient, the C/EBP δ expression levels are maintained for a longer period of time [25,53,112,180,181]. This is achieved, at least in part, through autoactivation [27,182,183]. Interestingly, analysis of the mouse, rat and ovine C/EBP δ promoters has also suggested the potential existence of species-specific mechanisms for autoregulation [27,182,183]. For example, the autoregulation of the rat C/EBP δ gene could not be seen with up to 6 kb of upstream promoter region, but instead required two C/EBP recognition sequences present at the 3' end of the gene [182]. In contrast, the 5' ends of the mouse and the ovine C/EBP δ genes are sufficient for auto-activation [27,183]. In the case of the ovine promoter, no putative C/EBP recognition sequences could be identified [27], thereby suggesting that, similar to the human C/EBP α gene [166], it also may be subject to indirect autoregulation.

C/EBP ϵ

The C/EBP ϵ gene promoter is transcribed by two alternative promoters, $p\alpha$ and $p\beta$, which, similar to those of several other myeloid-cell-specific genes, contains no TATAAA box, but have a number of purine-rich stretches with multiple sites for the ets family of transcriptional regulators [29–31]. The promoter also contains a functional retinoic acid-responsive element that mediates inducibility by the corresponding ligand [155].

C/EBP ζ

C/EBP ζ was first identified on the basis of its induced expression in response to growth arrest and DNA damage [32]. Such an inducibility was also seen in transfection assays using the –778/+21 promoter fragment from the hamster gene [32]. The activator protein-1 (AP-1) site present between –250 and –225 was found to be essential for this response and also played a role in the induction by oxidative stress [156]. Exposure of the cells to either oxidants or UVC irradiation was also shown to stimulate the binding of the c-Jun-c-Fos heterodimer to this element [156]. However, conflicting results have been obtained in relation to the induction of the endogenous C/EBP ζ mRNA and protein by UVC irradiation [157]. Because the expression of the C/EBP ζ gene is induced by a range of cellular stresses [39,133–135, 156–163], it is possible that differences in conditions for culturing of the cells may have been responsible for the discrepancies that exist. More recently, Schmitt-Ney and Habener [157] have re-examined the UVC response and shown that whereas the promoter is activated strongly in transfected cells, only a modest and transient increase in endogenous gene expression is observed. In addition, the induction of C/EBP ζ expression in response to other cellular stresses is markedly attenuated by prior exposure of the cells to UVC [157]. The target of such an inhibitory effect of UVC on C/EBP ζ expression is located in the first exon of the gene, a 5'-untranslated region that is highly conserved between different species [157].

Table 3 Regulators of the C/EBP family

Further abbreviations: DBA, DNA-binding activity; NL, nuclear localization; AP, activation potential. For any still-undefined abbreviations, see the abbreviations footnote.

Gene	Level	Activators	Repressors	References
C/EBP α	mRNA	Thyroid hormone, thiazolidinediones	EGF, glucocorticoid, growth hormone, IFN- γ , IL-1, IL-6, insulin, LPS, TNF- α	[19,25,112,114,140–143]
C/EBP β	DBA		EGF, PKC activators	[140,141,144]
	mRNA	cAMP, glucagon, glucocorticoid, growth hormone, IFN- γ , IL-1, IL-6, LPS, noradrenaline, NGF, VIP		[7,23,62,92,112–115,128,145–147]
C/EBP δ	NL	cAMP, LPS, TNF- α , TPA	TNF- α	[21,148–150]
	AP	Ca ²⁺ , IL-6, MAPK activators, PKC activators	Insulin	[8,53,125,151–153]
	DBA	TGF- β , EGF		[141]
C/EBP ϵ	mRNA	Glucocorticoid, growth hormone, IFN- γ , IL-1, IL-6, insulin, LPS, noradrenaline, PDGF, TNF- α , VIP		[25,53,92,112–115,145,147]
	NL	cAMP, TNF- α		[148,154]
C/EBP ζ	mRNA	Retinoic acid		[155]
C/EBP ζ	mRNA	Amino acid deprivation, agents that cause ER-, nutrient- and oxidative stress, Ca ²⁺ , nitric oxide, LPS, prostaglandin A ₂ , UV light		[32,33,39,133–135,156–163]
	AP	Cellular stress		[132]

The promoter region of the C/EBP ζ gene contains a conserved C/EBP recognition sequence, located between positions –339 to –320, that plays an important role in its induction during the APR in hepatocytes [158] (see below), and in response to sodium arsenite treatment of rat pheochromocytoma PC12 cells [159]. C/EBP ζ mRNA expression is induced by arsenite in a biphasic manner: a rapid increase during the first 4 h of treatment, a transient decline at 6–8 h, and a pronounced induction thereafter [159]. A closer examination of the C/EBP recognition site shows that it is a composite element with one half of each palindromic sequence comprising of an optimal C/EBP-binding motif and an activating transcription factor (ATF)/CRE variant site [159]. This C/EBP–ATF composite site interacts with both C/EBP β and ATF family members [159]. In addition, time-dependent changes in the interaction of ATF members with the composite site are seen following exposure of the cells to arsenite: binding of the activator ATF-4 at 2 h, when C/EBP ζ mRNA levels are increasing, and enhanced binding of the repressor ATF-3 at 6 h, when the levels are declining [159]. Thus the arsenite-induced biphasic induction of C/EBP ζ expression in PC12 cells involves the sequential binding of multiple factors to the C/EBP–ATF composite site [159].

Amino acid limitation also induces the expression of C/EBP ζ at both the transcriptional and post-transcriptional levels [160]. The decreasing amino acid concentration by itself can induce C/EBP ζ expression independently of a cellular stress produced by an inhibition of protein synthesis [162]. This induction of C/EBP ζ is mediated through the phosphoinositide 3-kinase (PI 3-kinase) and mammalian target of rapamycin (mTOR) signalling pathways that converge on the amino-acid-responsive element present between position –310 and –302 [161,162,184]. This regulatory sequence is related to the C/EBP–ATF-2 composite site and interacts with ATF-2 [162]. The precise mechanisms through which amino acid starvation regulates ATF-2 remains to be determined, but does not occur through any changes in its DNA-binding activity [162].

More recently, the –75 to –104 region of the C/EBP ζ promoter has been shown to be essential for both the constitutive and the ER-stress-inducible expression [163]. The ER stress-responsive element contains two overlapping regions that share sequence homology with similar regulatory sites identified in the glucose-regulated protein 78 (GRP78), GRP94, protein

disulphide-isomerase and calreticulin gene promoters [163]. The transcription factor NF-Y interacts with the two regulatory regions and mediates both constitutive and ER-stress-inducible activation of gene transcription [163].

Translation

Production of different C/EBP α and - β polypeptides by alternative use of initiation codons represents a major form of translational control in the regulation of the C/EBP family [18,48–50]. As detailed above, two distinct isoforms of C/EBP α , 42 kDa and 30 kDa, can be produced from a single mRNA (Figure 2) [49–50]. In contrast with the 42 kDa form, the 30 kDa polypeptide has a lower transactivation potential and lacks anti-mitotic activity [49,50]. The ratio of the two isoforms has been seen to change during both adipocyte differentiation and hepatocyte development [49,50], thereby raising the possibility of an important regulatory role. Similarly, in the case of C/EBP β , the ratio of the activator LAP form to the repressor LIP polypeptide has been shown to increase under a number of conditions, and such changes are likely to be functionally important [48]. For example, the expression of LIP is elevated in breast cancer, and this is consistent with its ability to induce epithelial cell proliferation and the formation of mammary hyperplasia [185]. It has been suggested that a LIP-mediated growth cascade may be susceptible to additional oncogenic hits, which could then result in the initiation and the progression of neoplasia [185].

The C/EBP α mRNA contains an evolutionary conserved short upstream open reading frame (uORF) that is a fixed distance of seven bases from the initiation codon that specifies for the full-length protein [186]. A short conserved uORF is also present in the C/EBP β mRNA [186]. Lincoln et al. [187] initially showed that such an uORF can act in *cis* to inhibit C/EBP translation as it is recognized readily by the translation machinery. It was proposed that, because of the potent differentiative and anti-mitotic activities of C/EBP α and, to a certain extent C/EBP β , such an inhibitory mechanism could operate to restrict the expression of the protein in cells that are undergoing differentiation, and also could have been responsible for the previously noted differences in the tissue expression profile of C/EBP α/β mRNA and the corresponding proteins [187]. More

recently, however, Calkhoven et al. [18] have shown instead that the integrity of the uORF is essential for the leaky ribosome scanning mechanism that leads to the production of different sized polypeptides [18]. The ratio of the different isoforms was found to be controlled by both an RNA-dependent protein kinase and the mTOR signalling pathway through the regulation of the eukaryotic translation initiation factors eIF-2 α and eIF-4E respectively [18].

Modulation of DNA-binding activity, transactivation potential and/or phosphorylation-mediated changes in nuclear localization

Phosphorylation plays a key role in the modulation of C/EBP β function. This isoform is normally a repressed factor in which negative regulatory regions mask its transactivation domains [40,46]. Such a repression is abolished by phosphorylation of the repression domain by several signal transduction pathways, including those mediated by oncogenic proteins [46]. The transactivation potential of C/EBP β is also induced by phosphorylation of Thr²³⁵ by a Ras/mitogen-activated protein kinase (MAPK) pathway [125,152], Ser¹⁰⁵ via protein kinase C (PKC) [153], and Ser²⁷⁶ by Ca²⁺/calmodulin-dependent protein kinase [151]. Phosphorylation can also suppress the activation potential of C/EBP β . For example, insulin inhibits transactivation by C/EBP β via the PI 3-kinase pathway [188]. In contrast, the PKA-mediated *in vitro* phosphorylation of a region between Ser¹⁷³ and Ser²²³, and Ser²⁴⁰, results in a suppression of C/EBP β DNA-binding activity [189]. Similarly, a PKC-mediated phosphorylation of Ser²⁴⁰ leads to a decrease in DNA binding [189]. On the other hand, the forskolin-induced activation of the cAMP/protein kinase A (PKA) signal-transduction pathway in rat PC12 cells results in the translocation of C/EBP β into the nucleus, where it then activates the transcription of the *c-fos* gene [149]. Antioxidant-induced nuclear translocation of C/EBP β in a colorectal cancer cell line DKO-1 is also mediated by a PKA-dependent phosphorylation, but of Ser²⁹⁹ [190]. Such phosphorylation-stimulated changes in the nucleo-cytoplasmic transport is also likely to be responsible, at least in part, for the regulation of C/EBP β during activation of macrophages [21,191] and the TNF α -mediated regulation of gene expression in hepatocytes [148,150].

Several studies have also suggested a potential role of phosphorylation in the regulation of C/EBP isoforms α , δ and ζ under certain conditions. For example, PKC can phosphorylate C/EBP α at several sites (Ser²⁴⁸, Ser²⁷⁷ and Ser²⁹⁹), and leads to an attenuation of its DNA-binding activity [144]. In addition, insulin can reduce the expression of genes in adipocytes, in part, due to a dephosphorylation-mediated degradation of C/EBP α [192] (see below). Furthermore, during cellular stress, C/EBP ζ undergoes an inducible phosphorylation at two adjacent serine residues (71 and 81) via the p38 MAPK pathway, which leads to enhanced transactivation potential of the factor [132]. Finally, studies on the regulation of the α_1 -acid glycoprotein and the serum amyloid A genes during the APR have shown that dephosphorylation of C/EBP δ results in an inhibition of its DNA binding activity [64]. Additionally, the transactivation potential of C/EBP δ was also found to be increased when hepatocytes were treated with cellular phosphatase inhibitors, such as okadaic acid and sodium orthovanadate [64].

Protein–protein interactions

The transactivation potential of the different C/EBP members differs; for example, C/EBP α is a stronger transcriptional activator than C/EBP β [8,9,53,60,71]. Because the different C/EBP members can form heterodimers under all intrafamilial

conditions [7–15], such interactions are likely to have a profound influence on the regulation of gene transcription. In addition, the C/EBPs can form protein–protein interactions with other bZIP and non-bZIP factors. For example, C/EBP β has been shown to interact with the p50 subunit of NF- κ B, CREB/ATF, AP-1, glucocorticoid receptor, hepatitis B virus X protein, and the retinoblastoma (Rb) protein [54–58]. Such heterodimers often have different transactivation potential and/or DNA binding specificity or affinity compared with the corresponding homodimer [54–58].

Regulation of the C/EBPs during adipocyte differentiation

Both C/EBP β and C/EBP δ mRNA is induced during the early phase of differentiation of pre-adipocyte cell lines in response to cAMP-elevating agents and glucocorticoids respectively [12,140]. The activation of these two members has been implicated, at least in part, in the later induction of C/EBP α gene transcription via a C/EBP recognition sequence present in the promoter region [65–67,88]. Once C/EBP α is induced, auto-activation ensures high expression levels, and thereby maintenance of the terminally differentiated state [65–67,88].

Although both C/EBP β and C/EBP δ are expressed at high levels at the start of the differentiation programme and throughout mitotic clonal expansion, C/EBP α is expressed much later [67]. Such a delayed expression of C/EBP α is crucial, because the protein is anti-mitotic and its premature expression would block the mitotic clonal expansion that is vital for differentiation [67]. Several mechanisms have been identified that prevent the premature early expression of C/EBP α . First, although both C/EBP β and C/EBP δ mRNAs are induced early in the differentiation programme, the DNA-binding activity is not acquired until the preadipocytes traverse the G₁-phase/S-phase checkpoint and initiate mitotic clonal expansion [193]. Concomitant with the acquisition of DNA-binding activity, C/EBP β/δ localize to centromeres by binding to multiple consensus C/EBP-binding sites in centromeric satellite DNA [193]. The precise mechanism that is involved in such acquisition of DNA-binding activity is currently unclear. However, at least C/EBP β has been shown to undergo phosphorylation concomitant with the acquisition of DNA-binding activity [67,193]. In addition, C/EBP ζ , which is expressed by growth-arrested pre-adipocytes, transiently sequesters C/EBP β/δ by heterodimerization [194]. As preadipocytes reach the S-phase, C/EBP ζ is down-regulated, thereby releasing C/EBP β/δ from the inhibitory constraint and, thereby, allowing activation of the C/EBP α gene [194]. In support of this proposition, up-regulation of C/EBP ζ with a protease inhibitor *N*-acetyl-Leu-Leu-norleucinal prevents activation of C/EBP β , expression of C/EBP α and adipogenesis [194]. Indeed, metabolic-stress conditions (e.g. low glucose) have been shown to inhibit adipogenesis by inducing the expression of C/EBP ζ [195]. Secondly, nuclear factor CUP, an isoform of AP-2 α , the expression of which is high in pre-adipocytes and declines drastically during differentiation, interacts with two repressive elements present upstream of the C/EBP α translation initiation codon, one located in the 5' flanking region and another in the 5' untranslated region [67,196,197]. Both these sites are required to maintain the promoter in an inactive state prior to differentiation [67,196,197]. Interestingly, the CUP regulatory element in the promoter region overlaps a Sp site (GT-element) to which Sp3 (or Sp1) interacts and, therefore, the binding by Sp3 and CUP/AP2- α is mutually exclusive [198]. Because Sp3 is a strong transcriptional activator and expressed throughout differentiation, it has been proposed that CUP/AP2- α may delay the access of Sp3 to the Sp regulatory element, and thereby

prevent the premature expression of C/EBP α [198]. Finally, the C/EBP site in the promoter region also overlaps with an Sp1 recognition sequence, with binding between the two factors being mutually exclusive [199]. Indeed, Sp1 has been demonstrated to block binding and activation by the C/EBPs both *in vitro* and in intact 3T3-L1 adipocytes [199]. Because Sp1 is expressed in pre-adipocytes and its levels decrease during differentiation, this could represent an additional mechanism for the repression of C/EBP α expression [199]. The decreased expression of Sp1 during differentiation has been shown to be due to a cAMP-mediated phosphorylation of the protein, which triggers its degradation [199].

Several factors have also been shown to modulate the expression of C/EBP isoforms in adipocytes. For example, insulin produces both a rapid dephosphorylation of the C/EBP α protein and reduces its mRNA expression [65,192]. C/EBP α is phosphorylated on about six sites in fully differentiated adipocytes, of which two, Thr²²² and Thr²²⁶, are de-phosphorylated in response to insulin treatment through inactivation of glycogen synthase kinase 3 [192]. In contrast, insulin induces the expression of the C/EBP β and C/EBP δ gene [65]. Glucocorticoids reciprocally regulate the expression of C/EBP α (decrease) and C/EBP δ (increase), mainly at the transcriptional level [140]. Finally, the expression of C/EBP α is attenuated by TNF- α , retinoic acid and TGF- β [142,200,201]. The precise mechanisms for cytokine action are currently unclear, with conflicting results being obtained with respect to mRNA expression [142,200]. However, retinoic acid blocks C/EBP α expression, and thus adipogenesis, by inhibiting C/EBP β -mediated transcription [201].

Regulation of the C/EBPs during the APR and liver regeneration

Both the APR and liver regeneration are characterized by a decrease in C/EBP α expression and an increase in that of C/EBP β and C/EBP δ [57,58,60,71]. Such reciprocal changes are mediated primarily through the actions of cytokines such as IL-6, IL-1 and TNF- α [57,58,60,71]. Analysis of the mouse C/EBP α promoter has shown that the LPS-mediated transcriptional suppression occurs through increased binding of the LIP isoform of C/EBP β to a C/EBP recognition sequence present in the promoter region [202]. The increased production of LIP under such conditions is achieved through regulation of translation by binding of the CUG repeat binding protein, CUGBP1, to recognition sites present between the first and second AUG codons of C/EBP β mRNA [202,203]. CUGBP1 binding activity is induced during the APR or liver regeneration, possibly in response to phosphorylation [202,203]. Although this model is potentially attractive and can explain the regulation of the mouse and the rat C/EBP α genes, it is not likely to be applicable to the human C/EBP α promoter, owing to the lack of a C/EBP recognition sequence [166]. In addition, Baer and Johnson [204] have shown recently that the truncated C/EBP β isoforms can be generated by *in vitro* proteolysis during isolation from cells, thereby indicating that any findings on gene regulation based on LIP have to be interpreted with caution and need to be confirmed *in vivo*.

The regulation of C/EBP β during the APR and liver regeneration is extremely complex, with control being exerted at both transcriptional and post-transcriptional levels [8,53,58,60,71]. Increased activity of CREB, which activates C/EBP β expression via binding to CRE-like sites in the promoter region, represents one important mechanism for transcriptional regulation, especially since an increase in CREB activity has been seen during liver regeneration [174]. These CRE-like elements are also required for the activation of C/EBP β transcription by IL-6, a

major mediator of the APR, via a novel pathway in which tethering of STAT-3 to a DNA-bound complex contributes to activation [175]. Post-transcriptional mechanisms, however, play the most prominent role in the regulation of C/EBP β during liver regeneration and the APR, and include increased production of LIP (see above), changes in nuclear-cytoplasmic transport, and phosphorylation-mediated modulation of DNA-binding activity and/or transactivation potential [8,53,60,71,202].

The transcription of the C/EBP δ gene is also activated by IL-6 and a number of pro-inflammatory factors during the APR [25,53,71,112]. The IL-6 induced expression is mediated via STAT-3, which interacts with a recognition sequence present in the proximal promoter region [180,181]. The activation of STAT-3 is transient, whereas the expression of C/EBP δ is more prolonged and therefore likely to be due to autoregulation [182]. Changes in the phosphorylation status of C/EBP δ also play a role in the regulation of at least some genes during the APR [64]. The expression of C/EBP δ mRNA is also induced during liver regeneration [57], although the mediator or the transcription factors that is responsible for such activation remains to be determined.

The C/EBP ζ gene is also induced during the APR, although its expression is delayed with respect to C/EBP β and C/EBP δ [158]. Such activation is mediated through the binding of C/EBP β or C/EBP δ to a recognition sequence present in the promoter region [158]. The precise reason(s) for the activation of C/EBP ζ during the APR is currently unclear, but may play a role in the curtailment of the response.

CONCLUDING REMARKS

Research carried out mainly in the last decade has revealed a pivotal role of the C/EBPs in numerous cellular responses, including proliferation, differentiation, apoptosis, control of metabolism and maintenance of specific functions. Studies on mice that are deficient in individual C/EBP members have clearly been instrumental in advancing our understanding of C/EBP function. However, the early demise of some knockout mice and functional redundancy between family members has clearly precluded the identification of the full range of different functions carried out by a specific gene. Conditional knockouts and analysis of mice that are deficient in more than one member will be necessary to resolve these issues. An important role of the C/EBPs in a number of disease conditions has also been determined, including the recent identification of specific gene mutations in patients (see, e.g. [101,102,117]). Thus further studies on the C/EBP family are essential to address fundamental questions in a number of areas, such as oncology, diabetes and haematopoiesis, and for the development of novel therapeutic strategies. We are only beginning to understand the transduction pathways that lead to the transcriptional and post-transcriptional regulation of the C/EBPs by specific signals. It is, therefore, important that further studies on this aspect are carried out using the full range of model systems that are available from different organisms given that species- and cell-type-specific differences in C/EBP regulation have been identified (e.g. [166]). Finally, the different C/EBP members have been shown to bind to an identical recognition sequence in the promoter regions of target genes, at least *in vitro* [57,58]. It is, however, possible that differences in the specificity and/or affinity to various binding sites may exist *in vivo*. This aspect needs to be addressed through the use of the chromatin immunoprecipitation technique [205], which has been employed recently to investigate the interaction of specific factors to recognition sequences in intact cells in the normal chromatin context.

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REFERENCES

- Johnson, P. F. and McKnight, S. L. (1989) Eukaryotic transcriptional regulatory proteins. *Annu. Rev. Biochem.* **58**, 799–839
- Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J. and McKnight, S. L. (1988) Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* **2**, 786–800
- Landschulz, W. H., Johnson, P. F. and McKnight, S. L. (1989) The DNA binding domain of the rat liver nuclear protein C/EBP is bipartite. *Science* **243**, 1681–1688
- Vinson, C. R., Sigler, P. B. and McKnight, S. L. (1989) Scissors-grip model for DNA recognition by a family of leucine zipper proteins. *Science* **246**, 911–916
- Agre, P., Johnson, P. F. and McKnight, S. L. (1989) Cognate DNA binding specificity retained after leucine zipper exchange between GCN4 and C/EBP. *Science* **246**, 922–926
- Hurst, H. C. (1995) Transcription factors 1: bZIP proteins. *Protein Profile* **2**, 101–168
- Akira, S., Isshiki, H., Sugita, H., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. and Kinoshita, T. (1990) A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *EMBO J.* **9**, 1897–1906
- Poli, V., Mancini, F. P. and Cortese, R. (1990) IL-6DBP, a nuclear protein involved in interleukin-6 signal transduction, defines a new family of leucine zipper proteins related to C/EBP. *Cell* **63**, 643–653
- Descombes, P., Chojkier, M., Lichtsteiner, S., Falvey, E. and Schibler, U. (1990) LAP, a novel member of the C/EBP gene family, encodes a liver-enriched transcriptional activator protein. *Genes Dev.* **4**, 1541–1551
- Chang, C. J., Chen, T. T., Lei, H. Y., Chen, D. S. and Lee, S. C. (1990) Molecular cloning of a transcription factor, AGP/EBP, that belongs to members of the C/EBP family. *Mol. Cell. Biol.* **10**, 6642–6653
- Roman, C., Platero, J. S., Shuman, J. and Calame, K. (1990) Ig/EBP-1: a ubiquitously expressed immunoglobulin enhancer binding protein that is similar to C/EBP and heterodimerizes with C/EBP. *Genes Dev.* **4**, 1404–1415
- Cao, Z., Umek, R. M. and McKnight, S. L. (1991) Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes Dev.* **5**, 1538–1552
- Williams, S. C., Cantwell, C. A. and Johnson, P. F. (1991) A family of C/EBP related proteins capable of forming covalently linked leucine zipper dimers *in vitro*. *Genes Dev.* **5**, 1553–1567
- Ron, D. and Habener, J. F. (1992) CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes Dev.* **6**, 439–453
- Rörth, P. and Montell, D. J. (1992) *Drosophila* C/EBP: a tissue-specific DNA-binding protein required for embryonic development. *Genes Dev.* **6**, 2299–2311
- Lyons, S. E., Shue, B. C., Lei, L., Oates, A. C., Zon, L. I. and Liu, P. P. (2001) A novel myeloid-restricted zebrafish CCAAT/enhancer-binding protein with a potent transcriptional activation domain. *Blood* **97**, 2611–2617
- Antonson, P. and Xanthopoulos, K. G. (1995) Molecular cloning, sequence, and expression of the human gene encoding CCAAT/enhancer binding protein α (C/EBP α). *Biochem. Biophys. Res. Commun.* **215**, 106–113
- Calkhoven, C. F., Muller, C. and Leutz, A. (2000) Translational control of C/EBP α and C/EBP β isoform expression. *Genes Dev.* **14**, 1920–1932
- Chen, Y., Hu, H. and Atkinson, B. G. (1994) Characterization and expression of C/EBP-like genes in the liver of *Rana catesbeiana* tadpoles during spontaneous and thyroid hormone-induced metamorphosis. *Dev. Genet.* **15**, 366–377
- Lyons, S. E., Shue, B. C., Lei, L., Oates, A. C., Zon, L. I. and Liu, P. P. (2001) Molecular cloning, genetic mapping, and expression analysis of four zebrafish C/EBP genes. *Gene* **281**, 43–51
- Katz, S., Kowenz-Leutz, E., Muller, C., Meese, K., Ness, S. A. and Leutz, A. (1993) The NF-M transcription factor is related to C/EBP β and plays a role in signal transduction, differentiation and leukemogenesis of avian myelomonocytic cells. *EMBO J.* **12**, 1321–1332
- Kousteni, S., Kockar, F. T., Sweeney, G. E. and Ramji, D. P. (1998) Characterization and developmental regulation of the *Xenopus laevis* CCAAT/enhancer binding protein β gene. *Mech. Dev.* **77**, 143–148
- Alberini, C. M., Ghirardi, M., Metz, R. and Kandel, E. R. (1994) C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in *Aplysia*. *Cell* **76**, 1099–1114
- Tucker, C. S., Hironi, I. and Aoki, T. (2002) Molecular cloning and expression of CCAAT/enhancer binding proteins in Japanese flounder *Paralichthys olivaceus*. *Dev. Comp. Immunol.* **26**, 271–282
- Kinoshita, S., Akira, S. and Kinoshita, T. (1992) A member of the C/EBP family, NF-IL6 β , forms a heterodimer and transcriptionally synergizes with NF-IL6. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1473–1476
- Kageyama, R., Sasai, Y. and Nakanishi, S. (1991) Molecular characterization of transcription factors that bind to the cAMP responsive region of the substance P precursor gene. cDNA cloning of a novel C/EBP related factor. *J. Biol. Chem.* **266**, 15525–15531
- Davies, G. E., Sabatakos, G., Cryer, A. and Ramji, D. P. (2000) The ovine CCAAT-enhancer binding protein δ gene: cloning, characterization, and species-specific autoregulation. *Biochem. Biophys. Res. Commun.* **271**, 346–352
- Antonson, P., Stellan, B., Yamanaka, R. and Xanthopoulos, K. G. (1996) A novel human CCAAT/enhancer binding protein gene, C/EBP ϵ , is expressed in cells of lymphoid and myeloid lineages and is localized on chromosome 14q11.2 close to the T-cell receptor α/δ locus. *Genomics* **35**, 30–38
- Chumakov, A. M., Grillier, I., Chumakova, E., Chih, D., Slater, J. and Koeffler, H. P. (1997) Cloning of the novel human myeloid-cell-specific C/EBP ϵ transcription factor. *Mol. Cell. Biol.* **17**, 1375–1386
- Yamanaka, R., Kim, G. D., Radomska, H. S., Lekstrom-Himes, J., Smith, L. T., Antonson, P., Tenen, D. G. and Xanthopoulos, K. G. (1997) CCAAT/enhancer binding protein ϵ is preferentially up-regulated during granulocyte differentiation and its functional versatility is determined by alternative use of promoters and differential splicing. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6462–6467
- Sabatakos, G., Kousteni, S., Cryer, A. and Ramji, D. P. (1998) Nucleotide sequence of ovine C/EBP ϵ gene. *J. Anim. Sci.* **76**, 2953–2954
- Luethy, J. D., Fargnoli, J., Park, J. S., Fornace Jr, A. J. and Holbrook, N. J. (1990) Isolation and characterization of the hamster gadd153 gene. Activation of promoter activity by agents that damage DNA. *J. Biol. Chem.* **265**, 16521–16526
- Park, J. S., Luethy, J. D., Wang, M. G., Fargnoli, J., Fornace Jr, A. J., McBride, O. W. and Holbrook, N. J. (1992) Isolation, characterization and chromosomal localization of the human GADD153 gene. *Gene* **116**, 259–267
- Osada, S., Yamamoto, H., Nishihara, T. and Imagawa, M. (1997) DNA binding specificity of the CCAAT/enhancer-binding protein transcription factor family. *J. Biol. Chem.* **271**, 3891–3896
- Tahirov, T. H., Inoue-Bungo, T., Morii, H., Fujikawa, A., Sasaki, M., Kimura, K., Shiina, M., Sato, K., Kumasaka, T., Yamamoto, M. et al. (2001) Structural analysis of DNA recognition by the AML1/Runx-1 Runt domain and its allosteric control by CBF β . *Cell* **104**, 755–767
- Tahirov, T. H., Sato, K., Ichikawa-Iwata, E., Sasaki, M., Inoue-Bungo, T., Shiina, M., Kitamura, K., Takata, S., Fujikawa, A., Morii, H. et al. (2002) Mechanism of c-Myb-C/EBP β cooperation from separated sites on a promoter. *Cell* **108**, 57–70
- Vinson, C. R., Hai, T. and Boyd, S. M. (1993) Dimerization specificity of the leucine zipper-containing bZIP motif on DNA binding: prediction and rational design. *Genes Dev.* **7**, 1047–1058
- Johnson, P. F. (1993) Identification of C/EBP basic region residues involved in DNA sequence recognition and half-site spacing preference. *Mol. Cell. Biol.* **13**, 6919–6930
- Ubeda, M., Wang, X. Z., Zinszner, H., Wu, I., Habener, J. F. and Ron, D. (1996) Stress-induced binding of the transcriptional factor CHOP to a novel DNA control element. *Mol. Cell. Biol.* **16**, 1479–1489
- Williams, S. C., Baer, M., Dillner, A. J. and Johnson, P. F. (1995) CRP2 (C/EBP β) contains a bipartite regulatory domain that controls transcriptional activation, DNA binding and cell specificity. *EMBO J.* **14**, 3170–3183
- Nerlov, C. and Ziff, E. B. (1994) Three levels of functional interaction determine the activity of CCAAT/enhancer binding protein- α on the serum albumin promoter. *Genes Dev.* **8**, 350–362
- Williamson, E. A., Xu, H. N., Gombart, A. F., Verbeek, W., Chumakov, A. M., Friedman, A. D. and Koeffler, H. P. (1998) Identification of transcriptional activation and repression domains in human CCAAT/enhancer-binding protein ϵ . *J. Biol. Chem.* **273**, 14796–14804
- Angerer, N. D., Du, Y., Nalbant, D. and Williams, S. C. (1999) A short conserved motif is required for repressor domain function in the myeloid-specific transcription factor CCAAT/enhancer binding protein ϵ . *J. Biol. Chem.* **274**, 4147–4154
- Tang, J.-G. and Koeffler, H. P. (2001) Structural and functional studies of CCAAT/enhancer-binding protein ϵ . *J. Biol. Chem.* **276**, 17739–17746
- Cooper, C., Henderson, A., Artandi, S., Avitahl, N. and Calame, K. (1995) Ig/EBP (C/EBP γ) is a transdominant negative inhibitor of C/EBP family of transcriptional activators. *Nucleic Acids Res.* **23**, 4371–4377
- Kowenz-Leutz, E., Twamley, G., Ansieau, S. and Leutz, A. (1994) Novel mechanism of C/EBP β (NF-M) transcriptional control: activation through derepression. *Genes Dev.* **8**, 2781–2791
- Pei, D. Q. and Shih, C. H. (1991) An “attenuator domain” is sandwiched by two distinct transactivation domains in the transcription factor C/EBP. *Mol. Cell. Biol.* **11**, 1480–1487

- 48 Descombes, P. and Schibler, U. (1991) A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. *Cell* **67**, 569–579
- 49 Ossipow, V., Descombes, P. and Schibler, U. (1993) CCAAT/enhancer binding protein mRNA is translated into multiple proteins with different transcription activation potentials. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8219–8223
- 50 Lin, F. T., MacDougald, O. A., Diehl, A. M. and Lane, M. D. (1993) A 30 kDa alternative translation product of the CCAAT/enhancer binding protein α message: transcriptional activator lacking antimetabolic activity. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9606–9610
- 51 Welm, A. L., Timochenko, N. A. and Darlington, G. J. (1999) C/EBP α regulates generation of C/EBP β isoforms through activation of specific proteolytic cleavage. *Mol. Cell. Biol.* **19**, 1695–1704
- 52 Lekstrom-Himes, J. A. (2001) The role of C/EBP ϵ in the terminal stages of granulocyte differentiation. *Stem Cells*. **19**, 125–133
- 53 Ramji, D. P., Vitelli, A., Tronche, F., Cortese, R. and Ciliberto, G. (1993) The two C/EBP isoforms, IL-6DBP/NF-IL6 and C/EBP δ /NF-IL6 β , are induced by IL-6 to promote acute phase gene transcription via different mechanisms. *Nucleic Acids Res.* **21**, 289–294
- 54 LeClair, K. P., Blonar, M. A. and Sharp, P. A. (1992) The p50 subunit of NF- κ B associates with the NF-IL6 transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8145–8149
- 55 Vallejo, M., Ron, D., Miller, C. P. and Habener, J. F. (1993) C/ATF, a member of the activating transcription factor family of DNA-binding proteins, dimerizes with CCAAT/enhancer-binding proteins and directs their binding to cAMP response elements. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4679–4683
- 56 Hsu, W., Kerppola, T. K., Chen, P. L., Curran, T. and Chen-Kiang, S. (1994) Fos and Jun repress transcriptional activation by NF-IL6 through association at the basic zipper region. *Mol. Cell. Biol.* **14**, 268–276
- 57 Lekstrom-Himes, J. and Xanthopoulos, K. G. (1998) Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J. Biol. Chem.* **273**, 28545–28548
- 58 Wedel, A. and Ziegler-Heitbrock, H. W. L. (1995) The C/EBP family of transcription factors. *Immunobiol.* **193**, 171–185
- 59 Lee, Y. H., Sauer, B., Johnson, P. F. and Gonzalez, F. J. (1997) Disruption of the C/EBP α gene in adult mouse liver. *Mol. Cell. Biol.* **17**, 6014–6022
- 60 Ramji, D. P., Cortese, R. and Ciliberto, G. (1993) Regulation of C-reactive protein, haptoglobin and hemopexin gene expression. In *Acute Phase Proteins: Molecular Biology, Biochemistry and Clinical Applications* (Mackiewicz, A., Kushner, M. D. and Baumann, H., eds.), pp. 162–242. CRC Press, Boca Raton, FL
- 61 Wang, N. D., Finegold, M. J., Bradley, A., Ou, C. N., Abdelsayed, S. V., Wilde, M. D., Taylor, L. R., Wilson, D. R. and Darlington, G. J. (1995) Impaired energy homeostasis in C/EBP α knockout mice. *Science* **269**, 1108–1112
- 62 Roesler, W. J. (2001) The role of C/EBP in nutrient and hormonal regulation of gene expression. *Annu. Rev. Nutr.* **21**, 141–165
- 63 Kimura, T., Christoffels, V. M., Chowdhury, S., Iwase, K., Matsuzaki, H., Mori, M., Lamers, W. H., Darlington, G. J. and Takiguchi, M. (1998) Hypoglycemia-associated hyperammonemia caused by impaired expression of ornithine cycle enzyme genes in C/EBP α knockout mice. *J. Biol. Chem.* **273**, 27505–27510
- 64 Ray, A. and Ray, B. K. (1994) Serum amyloid A gene expression under acute-phase conditions involves participation of inducible C/EBP β and C/EBP δ and their activation by phosphorylation. *Mol. Cell. Biol.* **14**, 4324–4332
- 65 Darlington, G. J., Ross, S. E. and MacDougald, O. A. (1998) The role of C/EBP genes in adipocyte differentiation. *J. Biol. Chem.* **273**, 30057–30060
- 66 Rosen, E. D., Walkey, C. J., Puigserver, P. and Spiegelman, B. M. (2000) Transcriptional regulation of adipogenesis. *Genes Dev.* **14**, 1293–1307
- 67 Lane, M. D., Tang, Q.-Q. and Jiang, M.-S. (1999) Role of CCAAT enhancer binding proteins (C/EBPs) in adipocyte differentiation. *Biochem. Biophys. Res. Commun.* **266**, 677–683
- 68 Mason, M. M., He, Y., Chen, H., Quon, M. J. and Reitman, M. (1998) Regulation of leptin promoter function by Sp1, C/EBP, and a novel factor. *Endocrinology (Baltimore)* **139**, 1013–1022
- 69 Hwang, C. S., Mandrup, S., MacDougald, O. A., Geiman, D. E. and Lane, M. D. (1996) Transcriptional activation of the mouse obese (*ob*) gene by CCAAT/enhancer binding protein α . *Proc. Natl. Acad. Sci. U.S.A.* **93**, 873–877
- 70 Elberg, G., Gimble, J. M. and Tsai, S. Y. (2000) Modulation of the murine peroxisome proliferator-activator receptor γ 2 promoter activity by CCAAT/enhancer binding proteins. *J. Biol. Chem.* **275**, 27815–27822
- 71 Poli, V. (1998) The role of C/EBP isoforms in the control of inflammatory and native immunity functions. *J. Biol. Chem.* **273**, 29279–29282
- 72 Yamanaoka, R., Barlow, C., Lekstrom-Himes, J., Castilla, L. H., Liu, P. P., Eckhaus, M., Decker, T., Wynshaw-Boris, A. and Xanthopoulos, K. G. (1997) Impaired granulopoiesis, myelodysplasia and early lethality in CCAAT/enhancer binding protein ϵ -deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13187–13192
- 73 Bretz, J. D., Williams, S. C., Baer, M., Johnson, P. F. and Schwartz, R. C. (1994) C/EBP-related protein 2 confers lipopolysaccharide-inducible expression of interleukin 6 and monocyte chemoattractant protein 1 to lymphoblastic cell line. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7306–7310
- 74 Williams, S. C., Du, Y., Schwartz, R. C., Weiler, S. R., Ortiz, M., Keller, J. R. and Johnson, P. F. (1998) C/EBP ϵ is a myeloid-specific activator of cytokine, chemokine, and macrophage-colony-stimulating factor receptor genes. *J. Biol. Chem.* **273**, 13493–13501
- 75 Pan, Z., Hetherington, C. J. and Zhang, D. E. (1999) CCAAT/enhancer-binding protein activates the CD14 promoter and mediates transforming growth factor β signaling in monocyte development. *J. Biol. Chem.* **274**, 23242–23248
- 76 Caivano, M., Gorgoni, B., Cohen, P. and Poli, V. (2001) The induction of cyclooxygenase-2 mRNA in macrophages is biphasic and requires both CCAAT enhancer-binding protein β (C/EBP β) and C/EBP δ transcription factors. *J. Biol. Chem.* **276**, 48693–48701
- 77 Zhang, D. E., Zhang, P., Wang, N. D., Hetherington, C. J., Darlington, G. J. and Tenen, D. G. (1997) Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein- α -deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 569–574
- 78 Zhang, P., Iwama, A., Datta, M. W., Darlington, G. J., Link, D. C. and Tenen, D. G. (1998) Upregulation of interleukin 6 and granulocyte colony-stimulating factor receptors by transcription factor CCAAT enhancer binding protein α (C/EBP α) is critical for granulopoiesis. *J. Exp. Med.* **188**, 1173–1184
- 79 Verbeek, W., Lekstrom-Himes, J., Park, D. J., Dang, P. M., Vuong, P. T., Kawano, S., Babior, B. M., Xanthopoulos, K. and Koeffler, H. P. (1999) Myeloid transcription factor C/EBP ϵ is involved in the positive regulation of lactoferrin gene expression in neutrophils. *Blood* **94**, 3141–3150
- 80 Lekstrom-Himes, J. and Xanthopoulos, K. G. (1999) CCAAT/enhancer binding protein ϵ is critical for effective neutrophil-mediated response to inflammatory challenge. *Blood* **93**, 3096–3105
- 81 Tavor, S., Vuong, P. T., Park, D. J., Gombart, A. F., Cohen, A. H. and Koeffler, H. P. (2002) Macrophage functional maturation and cytokine production are impaired in C/EBP ϵ -deficient mice. *Blood* **99**, 1794–1801
- 82 Flodby, P., Barlow, C., Kylefjord, H., Ahrlund-Richter, L. and Xanthopoulos, K. G. (1996) Increased hepatic cell proliferation and lung abnormalities in mice deficient in CCAAT/enhancer binding protein α . *J. Biol. Chem.* **271**, 24753–24760
- 83 Cassel, T. N., Nordlund-Moller, L., Andersson, O., Gustafsson, J. A. and Nord, M. (2000) C/EBP α and C/EBP δ activate the clara cell secretory protein gene through interaction with two adjacent C/EBPbinding sites. *Am. J. Respir. Cell. Mol. Biol.* **22**, 469–480
- 84 Sugahara, K., Iyama, K.-I., Kimura, T., Sano, K., Darlington, G. J., Akiba, T. and Takiguchi, M. (2001) Mice lacking CCAAT/enhancer-binding protein- α show hyperproliferation of alveolar type II cells and increased surfactant protein mRNAs. *Cell Tissue Res.* **306**, 57–63
- 85 Zhu, S., Oh, H. S., Shim, M., Sterneck, E., Johnson, P. F. and Smart, R. C. (1999) C/EBP β modulates the early events of keratinocyte differentiation involving growth arrest and keratin 1 and keratin 10 expression. *Mol. Cell. Biol.* **19**, 7181–7190
- 86 Robinson, G. W., Johnson, P. F., Hennighausen, L. and Sterneck, E. (1998) The C/EBP β transcription factor regulates epithelial cell proliferation and differentiation in the mammary gland. *Genes Dev.* **12**, 1907–1916
- 87 Seagroves, T. N., Krnacik, S., Raught, B., Gay, J., Burgess-Beusse, B., Darlington, G. J. and Rosen, J. M. (1998) C/EBP β , but not C/EBP α , is essential for ductal morphogenesis, lobuloalveolar proliferation, and functional differentiation in the mouse mammary gland. *Genes Dev.* **12**, 1917–1928
- 88 Christy, R. J., Haestner, K. H., Geiman, D. E. and Lane, M. D. (1991) CCAAT/enhancer binding protein gene promoter: binding of nuclear factors during differentiation of 3T3-L1 preadipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2593–2597
- 89 Wu, Z., Bucher, N. L. and Farmer, S. R. (1996) Induction of peroxisome proliferator-activated receptor γ during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBP β , C/EBP δ , and glucocorticoids. *Mol. Cell. Biol.* **16**, 4128–4136
- 90 Freytag, S. O., Paielli, D. L. and Gilbert, J. D. (1994) Ectopic expression of the CCAAT/enhancer-binding protein α promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes Dev.* **15**, 1654–1663
- 91 Lin, F. T. and Lane, M. D. (1994) CCAAT/enhancer binding protein α is sufficient to initiate the 3T3-L1 adipocyte differentiation program. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8757–8761
- 92 Yeh, W.-C., Cao, Z., Classon, M. and McKnight, S. L. (1994) Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev.* **9**, 168–181
- 93 Lin, F. T. and Lane, M. D. (1992) Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. *Genes Dev.* **6**, 533–544

- 94 Tanaka, T., Yoshida, N., Kishimoto, T. and Akira, S. (1997) Defective adipocyte differentiation in mice lacking the C/EBP β and/or C/EBP δ gene. *EMBO J.* **16**, 7432–7443
- 95 Linhart, H. G., Ishimura-Oka, K., DeMayo, F., Kibe, T., Repka, D., Poindexter, B., Bick, R. J. and Darlington, G. J. (2001) C/EBP α is required for differentiation of white, but not brown, adipose tissue. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12532–12537
- 96 Wu, Z., Rosen, E. D., Brun, R., Hauser, S., Adelmant, G., Troy, A. E., McKeon, C., Darlington, G. J. and Spiegelman, B. M. (1999) Cross-regulation of C/EBP α and PPAR γ controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol. Cell.* **3**, 151–158
- 97 Rosen, E. D., Hsu, C.-H., Wang, X., Sakai, S., Freeman, M. W., Gonzalez, F. J. and Spiegelman, B. M. (2002) C/EBP α induces adipogenesis through PPAR γ : a unified pathway. *Genes Dev.* **16**, 22–26
- 98 Scott, L. M., Civin, C. I., Rorth, P. and Friedman, A. D. (1992) A novel temporal expression pattern of three C/EBP family members in differentiating myelomonocytic cells. *Blood* **80**, 1725–1735
- 99 Morosetti, R., Park, D. J., Chumakov, A. M., Grillier, I., Shiohara, M., Gombart, A. F., Nakamaki, T., Weinberg, K. and Koefler, H. P. (1997) A novel, myeloid transcription factor, C/EBP ϵ , is upregulated during granulocytic, but not monocytic, differentiation. *Blood* **90**, 2591–2600
- 100 Natsuka, S., Akira, S., Nishio, Y., Hashimoto, S., Sugita, T., Isshiki, H. and Kishimoto, T. (1992) Macrophage differentiation-specific expression of NF-IL6, transcription factor for IL-6. *Blood* **79**, 460–466
- 101 Lekstrom-Himes, J. A., Dorman, S. E., Kopar, P., Holland, S. M. and Gallin, J. I. (1999) Neutrophil-specific granule deficiency results from a novel mutation with loss of function of the transcription factor CCAAT/enhancer binding protein ϵ . *J. Exp. Med.* **189**, 1847–1852
- 102 Gombart, A. F., Shiohara, M., Kwok, S. H., Agematsu, K., Komiyama, A. and Koefler, H. P. (2001) Neutrophil-specific granule deficiency: homozygous recessive inheritance of a frameshift mutation in the gene encoding transcription factor CCAAT/enhancer binding protein- ϵ . *Blood* **97**, 2561–2567
- 103 Tanaka, T., Akira, S., Yoshida, K., Uemoto, M., Yoneda, Y., Shirafuji, N., Fujiwara, H., Suematsu, S., Yoshida, N. and Kishimoto, T. (1995) Targeted disruption of the NF-IL6 gene discloses its essential role in bacterial killing and tumor cytotoxicity by macrophages. *Cell* **80**, 353–361
- 104 Screpanti, I., Romani, L., Musiani, P., Modesti, A., Fattori, E., Lazzaro, D., Sellitto, C., Scarpa, S., Bellavia, D., Lattanzio, G. et al. (1995) Lymphoproliferative disorder and imbalanced T-helper response in C/EBP β -deficient mice. *EMBO J.* **14**, 1932–1941
- 105 Sterneck, E., Tessarollo, L. and Johnson, P. F. (1997) An essential role for C/EBP β in female reproduction. *Genes Dev.* **11**, 2153–2162
- 106 Cortes-Canteli, M., Pignatelli, M., Santos, A. and Perez-Castillo, A. (2002) CCAAT/enhancer binding protein β plays a regulatory role in differentiation and apoptosis of neuroblastoma cells. *J. Biol. Chem.* **277**, 5460–5467
- 107 Chandrasekaran, C. and Gordon, J. I. (1993) Cell lineage-specific and differentiation-dependent patterns of CCAAT/enhancer binding protein α in the gut epithelium of normal and transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8871–8875
- 108 Tomizawa, M., Garfield, S., Factor, V. and Xanthopoulos, K. G. (1998) Hepatocytes deficient in CCAAT/enhancer binding protein α (C/EBP α) exhibit both hepatocyte and biliary epithelial cell character. *Biochem. Biophys. Res. Commun.* **249**, 1–5
- 109 McKnight, S. L., Lane, M. D. and Gluecksohn-Waelsch, S. (1989) Is CCAAT/enhancer-binding protein a central regulator of energy metabolism? *Genes Dev.* **3**, 2021–2024
- 110 Liu, S., Croninger, C., Arizmendi, C., Harada-Shiba, M., Ren, J., Poli, V., Hanson, R. W. and Friedman, J. E. (1999) Hypoglycemia and impaired hepatic glucose production in mice with a deletion of the C/EBP β gene. *J. Clin. Invest.* **103**, 207–213
- 111 Croninger, C. M., Millward, C., Yang, J., Kawai, Y., Arinze, I. J., Liu, S., Harada-Shiba, M., Chakravarthy, K., Friedman, J. E., Poli, V. and Hanson, R. W. (2001) Mice with a deletion in the gene for CCAAT/enhancer-binding protein β have an attenuated response to cAMP and impaired carbohydrate metabolism. *J. Biol. Chem.* **276**, 629–638
- 112 Alam, T., An, M. R. and Papaconstantinou, J. (1992) Differential expression of three C/EBP isoforms in multiple tissues during the acute phase response. *J. Biol. Chem.* **267**, 5021–5024
- 113 Cardinaux, J. R., Allaman, I. and Magistretti, P. J. (2000) Pro-inflammatory cytokines induce the transcription factors C/EBP β and C/EBP δ in astrocytes. *Glia* **29**, 91–97
- 114 Tengku-Muhammad, T. S., Hughes, T. R., Ranki, H., Cryer, A. and Ramji, D. P. (2000) Differential regulation of macrophage CCAAT/enhancer binding protein isoforms by lipopolysaccharide and cytokines. *Cytokine* **12**, 1430–1436
- 115 Granger, R. L., Hughes, T. R. and Ramji, D. P. (2000) Stimulus- and cell-type-specific regulation of CCAAT/enhancer binding protein isoforms in glomerular mesangial cells by lipopolysaccharide and cytokines. *Biochim. Biophys. Acta* **1510**, 171–179
- 116 Hendricks-Taylor, L. R. and Darlington, G. J. (1995) Inhibition of cell proliferation by C/EBP α occurs in many cell types, does not require the presence of p53 or Rb and is not affected by the large T antigen. *Nucleic Acids Res.* **23**, 4726–4733
- 117 Pabst, T., Mueller, B. U., Zhang, P., Radomska, H. S., Narravulu, S., Schnittger, S., Behre, G., Hiddemann, W. and Tenen, D. G. (2001) Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein- α (C/EBP α), in acute myeloid leukaemia. *Nat. Genet.* **27**, 263–270
- 118 McKnight, S. L. (2001) McBindall – A better name for CCAAT/enhancer binding proteins? *Cell* **107**, 259–261
- 119 Timchenko, N. A., Harris, T. E., Wilde, M., Bilyeu, T. A., Burgess-Beusse, B. L., Finegold, M. J. and Darlington, G. J. (1997) CCAAT/enhancer binding protein α regulates p21 protein and hepatocyte proliferation in newborn mice. *Mol. Cell. Biol.* **17**, 7353–7361
- 120 Chen, P. L., Riley, D., Chen, Y. and Lee, W. H. (1996) Retinoblastoma protein positively regulates terminal differentiation through direct interaction with C/EBPs. *Genes Dev.* **10**, 2794–2804
- 121 Timchenko, N. A., Wilde, M. and Darlington, G. J. (1999) C/EBP α regulates formation of S-phase-specific E2F-p107 complexes in livers of newborn mice. *Mol. Cell. Biol.* **19**, 2936–2945
- 122 Wang, H., Iakova, P., Wilde, M., Welm, A., Goode, T., Roesler, W. J. and Timchenko, N. A. (2001) C/EBP α arrests cell proliferation through direct inhibition of cdk2 and cdk4. *Mol. Cell.* **8**, 817–824
- 123 Wang, H., Goode, T., Iakova, P., Albrecht, J. H. and Timchenko, N. A. (2002) C/EBP α triggers proteasome-dependent degradation of cdk4 during growth arrest. *EMBO J.* **21**, 930–941
- 124 Greenbaum, L. E., Li, W., Cressman, D. E., Peng, Y., Ciliberto, G., Poli, V. and Taub, R. (1998) CCAAT enhancer-binding protein β is required for normal hepatocyte proliferation in mice after partial hepatectomy. *J. Clin. Invest.* **102**, 996–1007
- 125 Zhu, S., Yoon, K., Sterneck, E., Johnson, P. F. and Smart, R. C. (2002) CCAAT/enhancer binding protein- β is a mediator of keratinocyte survival and skin tumorigenesis involving oncogenic ras signalling. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 207–212
- 126 Buck, M., Poli, V., vander Geer, P., Chojkier, M. and Hunter, T. (1999) Phosphorylation of rat serine 105 or mouse threonine 217 in C/EBP β is required for hepatocyte proliferation induced by TGF α . *Mol. Cell.* **4**, 1087–1092
- 127 Buck, M., Poli, V., Hunter, T. and Chojkier, M. (2001) C/EBP β phosphorylation by RSK creates a functional XED caspase inhibitory box critical for cell survival. *Mol. Cell.* **8**, 807–816
- 128 Sterneck, E. and Johnson, P. F. (1998) CCAAT/enhancer binding protein β is a neuronal transcriptional regulator activated by nerve growth factor receptor signaling. *J. Neurochem.* **70**, 2424–2433
- 129 Yukawa, K., Tanaka, T., Tsuji, S. and Akira, S. (1998) Expression of CCAAT/enhancer-binding proteins β and δ and their activities are intensified by cAMP signaling as well as Ca²⁺/calmodulin kinases activation in hippocampal neurons. *J. Biol. Chem.* **273**, 31345–31351
- 130 Taubenfeld, S. M., Wiig, K. A., Monti, B., Dolan, B., Pollonini, G. and Alberini, C. M. (2001) Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein β and δ co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term memory consolidation. *J. Neurosci.* **21**, 84–91
- 131 Sterneck, E., Paylor, R., Jackson-Lewis, V., Libbey, M., Przedborski, S., Tessarollo, L., Crawley, J. N. and Johnson, P. F. (1998) Selectively enhanced contextual fear conditioning in mice lacking the transcriptional regulator CCAAT/enhancer binding protein δ . *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10908–10913
- 132 Wang, X. Z. and Ron, D. (1996) Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* **272**, 1347–1349
- 133 Zinszner, H., Kuroda, M., Wang, X.-Z., Batchvarova, N., Lightfoot, R. T., Remotti, H., Stevens, J. L. and Ron, D. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.* **12**, 982–995
- 134 Oyadomari, S., Takeda, K., Takiguchi, M., Gotoh, T., Matsumoto, M., Wada, I., Akira, S., Araki, E. and Mori, M. (2001) Nitric-oxide-induced apoptosis in pancreatic β cells is mediated by the endoplasmic reticulum stress pathway. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10845–10850
- 135 Oyadomari, S., Koizumi, A., Takeda, K., Gotoh, T., Akira, S., Araki, E. and Mori, M. (2002) Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J. Clin. Invest.* **109**, 525–532

- 136 Crozat, A., Aman, P., Mandahl, N. and Ron, D. (1993) Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature (London)* **363**, 640–644
- 137 Rabbits, T. H., Forster, A., Larson, R. and Nathan, P. (1993) Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nat. Genet.* **4**, 175–180
- 138 Barone, M. V., Crozat, A. Y., Tabae, L., Philipson, L. and Ron, D. (1994) CHOP (GADD153) and TLS-CHOP, differ in their ability to induce G₁/S arrest. *Genes Dev.* **8**, 453–464
- 139 Kaisho, T., Tsutsui, H., Tanaka, T., Tsujimura, T., Takeda, K., Kawai, T., Yoshida, N., Nakanishi, K. and Akira, S. (1999) Impairment of natural killer cytotoxic activity and interferon γ production in CCAAT/enhancer binding protein γ -deficient mice. *J. Exp. Med.* **11**, 1573–1581
- 140 MacDougald, O. A., Cornelius, P., Lin, F. T., Chen, S. S. and Lane, M. D. (1994) 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.* **269**, 19041–19047
- 141 Rana, B., Xie, Y., Mischoulon, D., Bucher, N. L. and Farmer, S. R. (1995) The DNA binding activity of C/EBP transcription factor is regulated in the G1 phase of the hepatocyte cell cycle. *J. Biol. Chem.* **270**, 18123–18132
- 142 Stephens, J. M. and Pekala, P. H. (1992) Transcriptional repression of the C/EBP α and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor- α . Regulation is coordinate and independent of protein synthesis. *J. Biol. Chem.* **267**, 13580–13584
- 143 Hemati, N., Erickson, R. L., Ross, S. E., Liu, R. and MacDougald, O. A. (1998) Regulation of CCAAT/enhancer binding protein α (C/EBP α) gene expression by thiazolidinediones in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* **244**, 20–25
- 144 Mahoney, C. W., Shuman, J., McKnight, S. L., Chen, H. C. and Huang, K. P. (1992) Phosphorylation of CCAAT-enhancer binding protein by protein kinase C attenuates site-selective DNA binding. *J. Biol. Chem.* **267**, 19396–19403
- 145 Cardinaux, J. R. and Magistretti, P. J. (1996) Vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide, and noradrenaline induce the transcription factors CCAAT/enhancer binding protein (C/EBP)- β and C/EBP δ in mouse cortical astrocytes: involvement in cAMP-regulated glycogen metabolism. *J. Neurosci.* **16**, 919–929
- 146 Matsuno, F., Chowdhury, S., Gotoh, T., Iwase, K., Matsuzaki, H., Takatsuki, K., Mori, M. and Takiguchi, M. (1996) Induction of C/EBP β gene by dexamethasone and glucagon in primary-cultured rat hepatocytes. *J. Biochem. (Tokyo)* **119**, 524–532
- 147 Clarkson, R. W., Chen, C. M., Harrison, S., Wells, C., Muscat, G. E. and Waters, M. J. (1995) Early responses of trans-activating factors to growth hormone in preadipocytes: differential regulation of CCAAT enhancer-binding protein- β (C/EBP β) and C/EBP δ . *Mol. Endocrinol.* **9**, 108–120
- 148 Yin, M., Yang, S. Q., Lin, H. Z., Lane, M. D., Chatterjee, S. and Diehl, A. M. (1996) Tumour necrosis factor α promotes nuclear localization of cytokine-inducible CCAAT/enhancer binding protein isoforms in hepatocytes. *J. Biol. Chem.* **271**, 17974–17978
- 149 Metz, R. and Ziff, E. (1991) cAMP stimulates the C/EBP related transcription factor rNF-IL-6 to trans-locate to the nucleus and induce c-fos transcription. *Genes Dev.* **5**, 1754–1766
- 150 Buck, M., Zhang, L., Halasz, N. A., Hunter, T. and Chojkier, M. (2001) Nuclear export of phosphorylated C/EBP β mediates the inhibition of albumin expression by TNF- α . *EMBO J.* **20**, 6712–6723
- 151 Wegner, M., Cao, Z. and Rosenfeld, M. G. (1992) Calcium-regulated phosphorylation within the leucine zipper of C/EBP β . *Science* **256**, 370–373
- 152 Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T. and Akira, S. (1993) Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2207–2211
- 153 Trautwein, C., Caelles, C., vander Geer, P., Hunter, T., Karin, M. and Chojkier, M. (1993) Transactivation by NF-IL6/LAP is enhanced by phosphorylation of its activation domain. *Nature (London)* **364**, 544–547
- 154 Billiard, J., Umayahara, Y., Wren, K., Centrella, M., McCarthy, T. L. and Rotwein, P. (2001) Regulated nuclear–cytoplasmic localization of CCAAT/enhancer-binding protein δ in osteoblasts. *J. Biol. Chem.* **276**, 15354–15361
- 155 Park, D. J., Chumakov, A. M., Vuong, P. T., Chih, D. Y., Gombart, A. F., Miller, Jr, W. H. and Koeffler, H. P. (1999) CCAAT/enhancer binding protein ϵ is a potential retinoid target gene in acute promyelocytic leukemia treatment. *J. Clin. Invest.* **103**, 1399–1408
- 156 Guyton, K. Z., Xu, Q. and Holbrook, N. J. (1996) Induction of the mammalian stress response gene GADD153 by oxidative stress: role of AP-1 element. *Biochem. J.* **314**, 547–554
- 157 Schmitt-Ney, M. and Habener, J. F. (2000) CHOP/GADD153 gene expression response to cellular stresses inhibited by prior exposure to ultraviolet light wavelength band C (UVC). Inhibitory sequence mediating the UVC response localized to exon 1. *J. Biol. Chem.* **275**, 40839–40845
- 158 Sylvester, S. L., Ap Rhys, C. M., Leiethy-Martindale, J. D. and Holbrook, N. J. (1994) Induction of GADD153, a CCAAT/enhancer-binding protein (C/EBP)-related gene, during the acute phase response in rats. Evidence for the involvement of C/EBPs in regulating its expression. *J. Biol. Chem.* **269**, 20119–20225
- 159 Fawcett, T. W., Martindale, J. L., Guyton, K. Z., Hai, T. and Holbrook, N. J. (1999) Complexes containing activating transcription factor (ATF)/cAMP-responsive-element-binding protein (CREB) interact with the CCAAT/enhancer-binding protein (C/EBP)/ATF composite site to regulate GADD153 expression during the stress response. *Biochem. J.* **339**, 135–141
- 160 Bruhat, A., Jousse, C., Wang, X. Z., Ron, D., Ferrara, M. and Fafournoux, P. (1997) Amino acid limitation induces expression of CHOP, a CCAAT/enhancer binding protein-related gene, at both transcriptional and post-transcriptional levels. *J. Biol. Chem.* **272**, 17588–17593
- 161 Bruhat, A., Jousse, C., Carraro, V., Reimold, A. M., Ferrara, M. and Fafournoux, P. (2000) Amino acids control mammalian gene transcription: activating transcription factor 2 is essential for the amino acid responsiveness of the CHOP promoter. *Mol. Cell. Biol.* **20**, 7192–7204
- 162 Fafournoux, P., Bruhat, A. and Jousse, C. (2000) Amino acid regulation of gene expression. *Biochem. J.* **351**, 1–12
- 163 Ubeda, M. and Habener, J. F. (2000) CHOP gene expression in response to endoplasmic reticular stress requires NFY interaction with different domains of a conserved DNA binding element. *Nucleic Acids Res.* **28**, 4987–4997
- 164 Tura-Kockar, F., Foka, P., Hughes, T. R., Kousteni, S. and Ramji, D. P. (2001) Analysis of the *Xenopus laevis* CCAAT-enhancer binding protein α gene promoter demonstrates species-specific differences in the mechanisms for both autoactivation and regulation by Sp1. *Nucleic Acids Res.* **29**, 362–372
- 165 Foka, P., Kousteni, S. and Ramji, D. P. (2001) Molecular characterization of the *Xenopus* CCAAT-enhancer binding protein β gene promoter. *Biochem. Biophys. Res. Commun.* **285**, 430–436
- 166 Timchenko, N., Wilson, D. R., Taylor, L. R., Abdelsayed, S., Wilde, M., Sawadogo, M. and Darlington, G. J. (1995) Autoregulation of the human C/EBP α gene by stimulation of upstream stimulating factor binding. *Mol. Cell. Biol.* **15**, 1192–1202
- 167 Legraverend, C., Antonson, P., Flodby, P. and Xanthopoulos, K. G. (1993) High level activity of the mouse CCAAT/enhancer binding protein (C/EBP α) gene promoter involves autoregulation and several ubiquitous transcription factors. *Nucleic Acids Res.* **21**, 1735–1742
- 168 Menendez-Hurtado, A., Santos, A. and Perez-Castillo, A. (2000) Characterization of the promoter region of the rat CCAAT/enhancer-binding protein α gene and regulation by thyroid hormone in rat immortalized brown adipocytes. *Endocrinology (Baltimore)* **141**, 4164–4170
- 169 Antonson, P., Pray, M. G., Jacobsson, A. and Xanthopoulos, K. G. (1995) Myc inhibits CCAAT/enhancer-binding protein- α gene expression in HIB-1B fibronoma cells through interactions with the core promoter regions. *Eur. J. Biochem.* **232**, 397–403
- 170 Li, L. H., Nerlov, C., Prendergast, G., MacGregor, D. and Ziff, E. B. (1994) c-myc represses transcription *in vivo* by a novel mechanism dependent on the initiator element and myc box II. *EMBO J.* **13**, 4070–4079
- 171 Chang, C. J., Shen, B. J. and Lee, S. C. (1995) Autoregulated induction of the acute-phase response transcription factor gene, agp/ebp. *DNA Cell Biol.* **14**, 529–537
- 172 Niehof, M., Kubicka, S., Zender, L., Manns, M. P. and Trautwein, C. (2001) Autoregulation enables different pathways to control CCAAT/enhancer binding protein β (C/EBP β) transcription. *J. Mol. Biol.* **309**, 855–868
- 173 Mink, S., Jaswal, S., Burk, O. and Klempner, K. H. (1999) The v-myc oncoprotein activates C/EBP β expression by stimulating an autoregulatory loop at the C/EBP β promoter. *Biochim. Biophys. Acta* **1447**, 175–184
- 174 Niehof, M., Manns, M. P. and Trautwein, C. (1997) CREB controls LAP/C/EBP β transcription. *Mol. Cell. Biol.* **17**, 3600–3613
- 175 Niehof, M., Street, K., Rakemann, T., Bischoff, S. C., Manns, M. P., Horn, F. and Trautwein, C. (2001) Interleukin-6-induced tethering of STAT3 to the LAP/C/EBP β promoter suggests a new mechanism of transcriptional regulation by STAT3. *J. Biol. Chem.* **276**, 9016–9027
- 176 Berrier, A., Siu, G. and Calame, K. (1998) Transcription of a minimal promoter from the NF-IL6 gene is regulated by CREB/ATF and Sp1 proteins in U937 promonocytic cells. *J. Immunol.* **161**, 2267–2275
- 177 McCarthy, T. L., Ji, C., Chen, Y., Kim, K. K., Imagawa, M., Ito, Y. and Centrella, M. (2000) Runt domain factor (Runx)-dependent effects on CCAAT/enhancer binding protein δ expression and activity in osteoblasts. *J. Biol. Chem.* **275**, 21746–21753
- 178 Hutt, J. A., O'Rourke, J. P. and DeWille, J. (2000) Signal transducer and activator of transcription 3 activates CCAAT/enhancer binding protein- δ gene transcription in G0 growth-arrested mouse mammary epithelial cells and in involuting mouse mammary gland. *J. Biol. Chem.* **275**, 29123–29131

- 179 Sabatakos, G., Davies, G. E., Grosse, M., Cryer, A. and Ramji, D. P. (1998) Expression of the genes encoding CCAAT-enhancer binding protein isoforms in the mouse mammary gland during lactation and involution. *Biochem. J.* **334**, 205–210
- 180 Yamada, T., Tobita, K., Osada, S., Nishihara, T. and Imagawa, M. (1997) CCAAT/enhancer binding protein δ gene expression is mediated by APRF/STAT3. *J. Biochem (Tokyo)*. **121**, 731–738
- 181 Cantwell, C. A., Sterneck, E. and Johnson, P. F. (1998) Interleukin-6-specific activation of the C/EBP δ gene in hepatocytes is mediated by Stat3 and Sp1. *Mol. Cell. Biol.* **18**, 2108–2117
- 182 Yamada, T., Tsuchiya, T., Osada, S., Nishihara, T. and Imagawa, M. (1998) CCAAT/enhancer binding protein δ gene expression is mediated through downstream binding sites. *Biochem. Biophys. Res. Commun.* **242**, 88–92
- 183 O'Rourke, J. P., Hutt, J. A. and DeWille, J. (1999) Transcriptional regulation of C/EBP δ in G(0) growth-arrested mouse mammary epithelial cells. *Biochem. Biophys. Res. Commun.* **262**, 696–701
- 184 Entingh, A. J., Law, B. K. and Moses, H. L. (2001) Induction of the C/EBP homologous protein (CHOP) by amino acid deprivation requires insulin-like growth factor I, phosphatidylinositol 3-kinase, and mammalian target of rapamycin signalling. *Endocrinology (Baltimore)* **142**, 221–228
- 185 Zahnow, C. A., Cardiff, R. D., Laucirica, R., Medina, D. and Rosen, J. M. (2001) A role for CCAAT/enhancer binding protein- β -liver-enriched inhibitory protein in mammary epithelial cell proliferation. *Cancer Res.* **61**, 261–269
- 186 Calkhoven, C. F., Bouwman, P. R., Snippe, L. and Ab, G. (1994) Translation start site multiplicity of the CCAAT/enhancer binding protein α mRNA is dictated by a small 5' open reading frame. *Nucleic Acids Res.* **22**, 5540–5547
- 187 Lincoln, A. J., Monczak, Y., Williams, S. C. and Johnson, P. F. (1998) Inhibition of CCAAT/enhancer binding protein α and β translation by upstream open reading frames. *J. Biol. Chem.* **273**, 9552–9560
- 188 Guo, S., Cichy, S. B., He, X., Yang, Q., Ragland, M., Ghosh, A. K., Johnson, P. F. and Unterman, T. G. (2001) Insulin suppresses transactivation by CCAAT/enhancer-binding protein β (C/EBP β). *J. Biol. Chem.* **276**, 8516–8523
- 189 Trautwein, C., van der Geer, P., Karin, M., Hunter, T. and Chojkier, M. (1994) Protein kinase A and C site-specific phosphorylations of LAP (NF-IL6) modulates its binding affinity to DNA recognition elements. *J. Clin. Invest.* **93**, 2554–2561
- 190 Chinery, R., Brockman, J. A., Dransfield, D. T. and Coffey, R. J. (1997) Antioxidant-induced nuclear translocation of CCAAT/enhancer-binding protein β . A critical role for protein kinase A-mediated phosphorylation of Ser299. *J. Biol. Chem.* **272**, 30356–30361
- 191 Baer, M., Williams, S. C., Dillner, A., Schwartz, R. C. and Johnson, P. F. (1998) Autocrine signals control CCAAT/enhancer binding protein β expression, localization, and activity in macrophages. *Blood* **92**, 4353–4365
- 192 Ross, S. E., Erickson, R. L., Hemati, N. and MacDougald, O. A. (1999) Glycogen synthase kinase 3 is an insulin-regulated C/EBP α kinase. *Mol. Cell. Biol.* **19**, 8433–8441
- 193 Tang, Q. Q. and Lane, M. D. (1999) Activation and centromeric localization of CCAAT/enhancer binding proteins during the mitotic clonal expansion of adipocyte differentiation. *Genes Dev.* **13**, 2231–2241
- 194 Tang, Q. Q. and Lane, M. D. (2000) Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer binding protein β during adipogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 12446–12450
- 195 Batchvarova, N., Wang, X. Z. and Ron, D. (1995) Inhibition of adipogenesis by the stress-induced protein CHOP (GADD153). *EMBO J.* **14**, 4654–4661
- 196 Tang, Q. Q., Jiang, M. S. and Lane, M. D. (1997) Repression of transcription mediated by dual elements in the CCAAT/enhancer binding protein α gene. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13571–13575
- 197 Jiang, M. S., Tang, Q. Q., McLenithan, J., Geiman, D., Shillinglaw, W., Henzel, W. J. and Lane, M. D. (1998) Derepression of the C/EBP α gene during adipogenesis: identification of AP-2 α as a repressor. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3467–3471
- 198 Jiang, M. S. and Lane, M. D. (2000) Sequential repression and activation of the CCAAT-enhancer binding protein- α (C/EBP α) gene during adipogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 12519–12523
- 199 Tang, Q. Q., Jiang, M. S. and Lane, M. D. (1999) Repressive effect of Sp1 on the C/EBP α gene promoter: role in adipocyte differentiation. *Mol. Cell. Biol.* **19**, 4855–4865
- 200 Ron, D., Brasier, A. R., McGehee, R. E. and Habener, J. F. (1992) Tumor necrosis factor-induced reversal of adipocytic phenotype of 3T3-L1 cells is preceded by a loss of nuclear CCAAT/enhancer binding protein C/EBP. *J. Clin. Invest.* **89**, 223–233
- 201 Schwarz, E. J., Reginato, M. J., Shao, D., Krakow, S. L. and Lazar, M. A. (1997) Retinoic acid blocks adipogenesis by blocking C/EBP β -mediated transcription. *Mol. Cell. Biol.* **17**, 1552–1561
- 202 Welm, A. L., Mackey, S. L., Timchenko, L. T., Darlington, G. J. and Timchenko, N. A. (2000) Translational induction of liver-enriched transcriptional inhibitory protein during acute phase response leads to repression of CCAAT/enhancer binding protein α mRNA. *J. Biol. Chem.* **275**, 27406–27413
- 203 Timchenko, N. A., Welm, A. L., Lu, X. and Timchenko, L. T. (1999) CUG repeat binding protein (CUGBP1) interacts with the 5' region of C/EBP β mRNA and regulates translation of C/EBP β isoforms. *Nucleic Acids Res.* **27**, 4517–4525
- 204 Baer, M. and Johnson, P. F. (2000) Generation of truncated C/EBP β isoforms by *in vitro* proteolysis. *J. Biol. Chem.* **275**, 26582–26590
- 205 Nal, B., Mohr, E. and Ferrier, P. (2001) Location analysis of DNA-bound proteins at the whole-genome level: untangling transcriptional regulatory networks. *Bioessays* **23**, 473–476

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