Int. J. Exp. Path. (1998), **79,** 369–391

***Focus on hepatology in Japan***

# The C/EBP family of transcription factors in the liver and other organs

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Received for publication 14 July 1998
Accepted for publication 20 July 1998

**Summary.** Members of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors are pivotal regulators of liver functions such as nutrient metabolism and its control by hormones, acute-phase response and liver regeneration. Recent progress in clarification of regulatory mechanisms for the C/EBP family members gives insight into understanding the liver functions at the molecular level.

*Keywords:* hepatocyte, gene expression, bZIP protein

Liver exhibits a variety of tissue-specific functions such as gluconeogenesis, urea synthesis, bile acid formation, cholesterol synthesis, plasma protein synthesis and drug metabolism. Liver is the most active organ in the acute-phase response characterizing the early stage of inflammation. While hepatocytes are normally resting in the $G_0$ phase of the cell cycle, they can be induced to proliferate, especially as an experimental procedure by partial hepatectomy. Resulting liver regeneration provides an excellent system for the study of cell proliferation *in vivo*.

Regulation at the transcription level serves as one of the most important steps to control these processes in the liver. As if to reflect complexity of hepatocyte-specific functions, a number of hepatocyte-selective transcription factors have been identified. These factors can be classified into five groups, based on the properties of their DNA-binding domain as follows: the CCAAT/enhancer-binding protein (C/EBP) family (Cao *et al*. 1991; Williams *et al*. 1991) is the prototype of transcription factors having a basic region/leucine zipper (bZIP) domain; albumin promoter site-D-binding protein (DBP) (Mueller *et al*. 1990), thyrotroph embryonic factor (TEF) (Drolet *et al*. 1991) and hepatocyte leukaemia factor (HLF) (Hunger *et al*. 1992; Inaba *et al*. 1992; Falvey *et al*. 1995) are members of another bZIP family characterized by a proline-and acidic-amino-acid-rich (PAR) domain adjacent to the basic region; hepatocyte nuclear factor-1 (HNF-1) (Frain *et al*. 1989; Baumhueter *et al*. 1990) contains an extralarge homeodomain; HNF-3 family members (Lai *et al*. 1990, 1991) have a winged helix domain; and HNF-4 (Sladek *et al*. 1990) belongs to the steroid receptor superfamily. Here, I focus on C/EBP family members, especially underlining their roles in dynamic processes of liver functions. Works from related fields are also referred.

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## Members of the C/EBP family

The first member of the C/EBP family, designated C/EBP$\alpha$, was originally purified from rat liver nuclear extracts as a heat-stable DNA-binding protein recognizing viral enhancer core sequences (Johnson *et al*. 1987) as well as the CCAAT box sequence (Graves *et al*. 1986), although today the most common CCAAT-box-binding activity is attributed to a different factor CBF/NF-Y (Mantovani *et al*. 1992; Bi *et al*. 1997). Molecular cloning of C/EBP$\alpha$ (Landschulz *et al*. 1988a) and subsequent structural analysis (Landschulz *et al*. 1988b,

1989) led to the discovery of a well–known dimerization interface the 'leucine zipper' juxtaposed to a DNA-binding surface the 'basic region', together being called the bZIP domain.

The second member C/EBP$\beta$ (Cao *et al*. 1991) was identified from several different approaches, and initially known by various names such as NF-IL6 (Akira *et al*. 1990), IL6-DBP (Poli *et al*. 1990), AGP/EBP (Chang *et al*. 1990), LAP (Descombes *et al*. 1990) and CRP2 (Williams *et al*. 1991). NF-IL6 was cloned as a factor binding to an IL1-responsive element of the IL-6 gene from human monocytes by screening an expression library with a double-stranded binding site DNA probe (South-western screening) (Akira *et al*. 1990). NF-IL6 was also shown to bind to regulatory regions of genes for hepatic acute-phase proteins (Akira *et al*. 1990). Similar approaches led to cloning of IL6-DBP as a rat factor binding to IL-6-responsive elements of several acute-phase genes (Poli *et al*. 1990), and AGP/EBP as a mouse C/EBP-like factor binding to a promoter site of an acute-phase protein $\alpha_1$-acid glycoprotein (Chang *et al*. 1990). A search for an additional factor binding to the C/EBP site of the albumin promoter led to isolation of a liver-enriched transcriptional activator protein (LAP) (Descombes *et al*. 1990). Screening for preadipocyte cDNA and genomic clones cross-hybridizing with DNA-binding domain sequences of C/EBP$\alpha$ resulted in isolation of the homologue named C/EBP$\beta$ (Cao *et al*. 1991) and CRP2 (Williams *et al*. 1991).

C/EBP$\gamma$, originally named Ig/EBP (Roman *et al*. 1990) and GPE1-BP (Nishizawa *et al*. 1991), was cloned by South-western screening using as probes the IgH enhancer element and the granulocyte colony-stimulating factor promoter element, respectively.

C/EBP$\delta$ (Cao *et al*. 1991) was isolated by several groups independently, and initially known also as CELF (Kageyama *et al*. 1991), CRP3 (Williams *et al*. 1991) and NF-IL6$\beta$ (Kinoshita *et al*. 1992). CELF was cloned by South-western screening of the rat barin cDNA library as a factor binding to a cAMP response element of the substance P gene (Kageyama *et al*. 1991). C/EBP$\delta$ (Cao *et al*. 1991) and CRP3 (Williams *et al*. 1991) were isolated by cross hybridization with C/EBP$\alpha$, as described above. NF-IL6$\beta$ was cloned by cross hybridization with DNA-binding domain sequences of NF-IL6 (C/EBP$\beta$) from a human placental genomic library (Kinoshita *et al*. 1992).

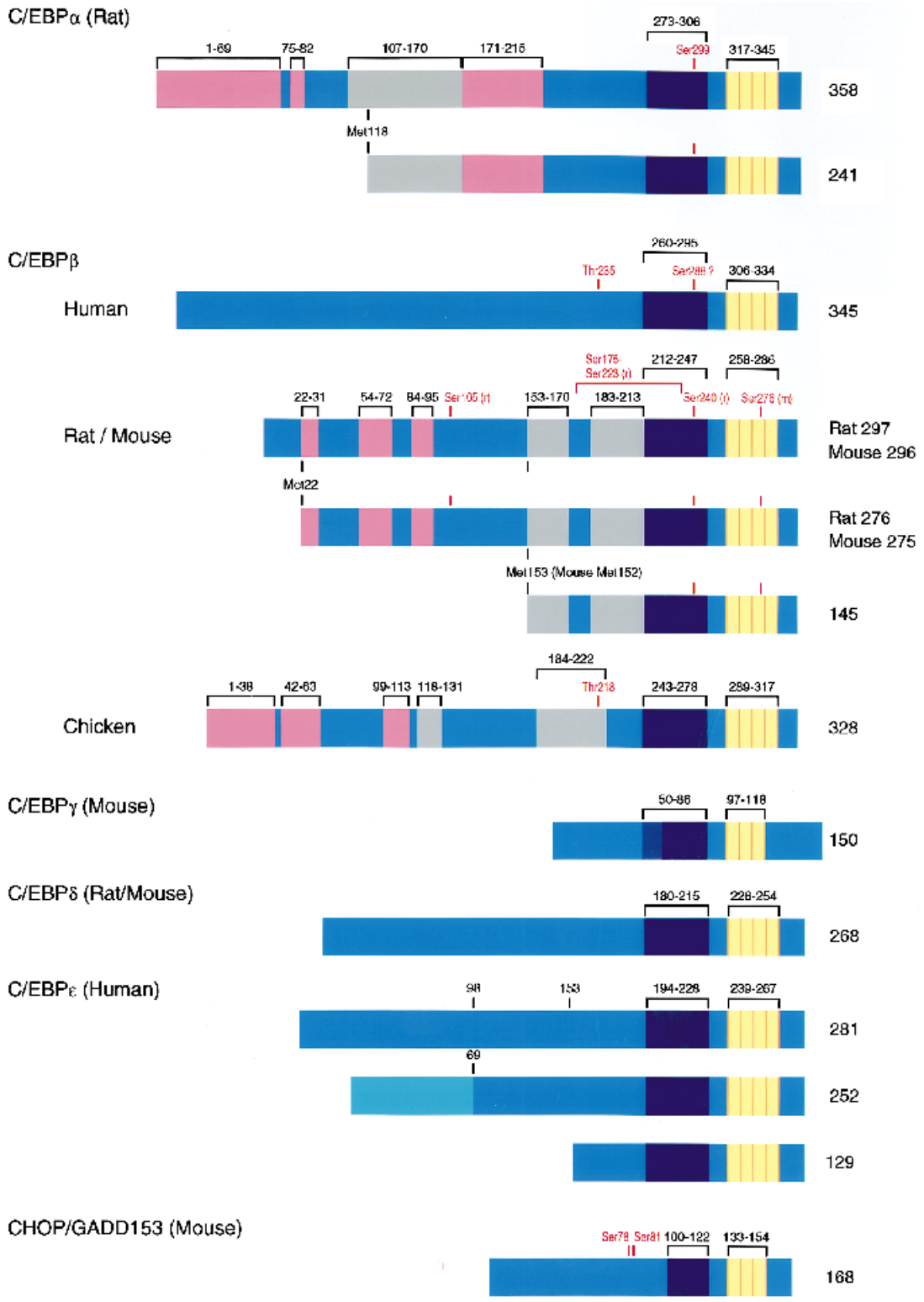
The rat gene for C/EBP$\varepsilon$, originally named CRP3 (Williams *et al*. 1991), was cloned by cross hybridization with C/EBP$\alpha$, without detection of its expression. Recently, it was shown that the human C/EBP$\varepsilon$ gene is expressed in granulocytes and lymphoid cells (Antonson *et al*. 1996; Chumakov *et al*. 1997; Yamanaka *et al*. 1997b).

The C/EBP-homologous protein CHOP was cloned as a factor interacting with the bZIP domain of C/EBP$\beta$ by screening an adipocyte cDNA expression library using a radiolabelled bZIP peptide as a probe (West-western or Far Western screening) (Ron & Habener 1992). The same factor had been formerly characterized as a product of the growth arrest-and DNA damage-inducible gene *gadd153* in Chinese hamster ovary (CHO) cells (Fornace *et al*. 1989).

Gene structures of C/EBP family members are relatively simple. Rodent genes for C/EBP$\alpha$ (Landschulz *et al*. 1988a), C/EBP$\beta$ (Descombes *et al*. 1990) and C/EBP$\delta$ (Cao *et al*. 1991; Williams *et al*. 1991) are intronless. The mouse C/EBP$\gamma$ gene consists of two exons (Nishizawa *et al*. 1991). The human C/EBP$\varepsilon$ gene is transcribed from two alternative promoters and shares the 3′ exon, consisting in total of three exons (Yamanaka *et al*. 1997b). The hamster (Luethy *et al*. 1990) and human (Park *et al*. 1992) CHOP/GADD153 gene span 5 kb and 3 kb, respectively, and both consist of four exons.

Chromosomal location was determined for human genes: C/EBP$\alpha$, 19q13.1; C/EBP$\beta$, 20q13 (Szpirer *et al*. 1991, 1992; Hendricks-Taylor *et al*. 1992); C/EBP$\delta$,

**Figure 1.** Array of C/EBPs, domain structures and phosphorylation sites. See the text for explanation. Coloured boxes represent following: pink, activation domain; grey, inhibition domain; dark blue, basic region; violet, deviated sequence of the basic region in C/EBP$\gamma$; yellow with orange bars, leucine zipper domain; light blue, alternative sequence of C/EBP$\varepsilon$. Red small bars and span indicate phosphorylation sites. References are as follows. C/EBP$\alpha$: amino acid sequences (Landschulz *et al*. 1988a; Lincoln *et al*. 1994); translation start sites (Lin *et al*. 1993; Ossipow *et al*. 1993); activation and inhibition domains (Friedman & McKnight 1990; Pei & Shih 1991; Nerlov & Ziff 1994; Nerlov & Ziff 1995); phosphorylation (Mahoney *et al*. 1992). Human C/EBP$\beta$: amino acid sequences (Akira *et al*. 1990); phosphorylation (Nakajima *et al*. 1993). Rat C/EBP$\beta$: amino acid sequences (Descombes *et al*. 1990; Williams *et al*. 1991); translation start sites (Descombes & Schibler 1991); activation and inhibition domains (Trautwein *et al*. 1995; Williams *et al*. 1995); phosphorylation (Trautwein *et al*. 1993; Trautwein *et al*. 1994). Mouse C/EBP$\beta$: amino acid sequences (Chang *et al*. 1990; Cao *et al*. 1991); phosphorylation (Wegner *et al*. 1992). Chicken C/EBP$\beta$ (NF-M): amino acid sequences (Burk *et al*. 1993; Katz *et al*. 1993); activation and inhibition domains, and phosphorylation (Kowenz-Leutz *et al*. 1994). C/EBP$\gamma$: amino acid sequences (Roman *et al*. 1990; Nishizawa *et al*. 1991). Rat C/EBP$\delta$: amino acid sequences (Kageyama *et al*. 1991). Mouse C/EBP$\delta$: amino acid sequences (Cao *et al*. 1991; Williams *et al*. 1991). C/EBP$\varepsilon$: amino acid sequences (Antonson *et al*. 1996; Yamanaka *et al*. 1997b). CHOP: amino acid sequences (Ron & Habener 1992); phosphorylation (Wang & Ron 1996).


C/EBPα (Rat)
1-69
75-82
107-170
171-215
273-306
Ser299
317-345
358
Met118
241
C/EBPβ
Human
Thr235
Ser288 ?
260-295
306-334
345
Rat / Mouse
Ser175-
Ser223 (r)
212-247
258-286
22-31
54-72
84-95
Ser105 (r)
153-170
183-213
Ser240 (r)
Ser275 (m)
Rat 297
Mouse 296
Met22
Rat 276
Mouse 275
Met153 (Mouse Met152)
145
Chicken
184-222
1-38
42-63
99-113
118-131
Thr218
243-278
289-317
328
C/EBPγ (Mouse)
50-86
97-118
150
C/EBPδ (Rat/Mouse)
180-215
228-254
268
C/EBPε (Human)
98
153
194-228
239-267
281
69
252
129
CHOP/GADD153 (Mouse)
Ser79
Ser81
100-122
133-154
168

8p11.2-p11.1 (Cleutjens *et al*. 1993; Wood *et al*. 1995); C/EBP$\varepsilon$, 14q11.2 (Antonson *et al*. 1996); CHOP/GADD153, 12q13.1-q13.2 (Park *et al*. 1992). Mouse genes are located as follows: C/EBP$\alpha$, 7 (Birkenmeier *et al*. 1989); C/EBP$\beta$, 2; C/EBP$\delta$, 16; C/EBP$\varepsilon$, 14 (Jenkins *et al*. 1995). C/EBP homologues were also found in invertebrates such as *Drosophila* (Montell *et al*. 1992; Rørth & Montell 1992; Rørth 1994) and *Aplysia* (Alberini *et al*. 1994).

## Complex array of protein species as activators or repressors

In spite of relatively simple architecture of genes of the C/EBP familly, a more complicated array of protein species can be generated (Figure 1). Production of three protein species from a single mRNA was described on rat C/EBP$\beta$ (Descombes & Schibler 1991) and later also two protein species on C/EBP$\alpha$ (Lin *et al*. 1993; Ossipow *et al*. 1993). In these cases, several AUG codons can be recognized as initiation codons by ribosomes, presumably because a fraction of ribosomes scanning mRNA ignore upstream AUG codons. The shortest form of C/EBP$\beta$, which lacks activation domains, functions as a negative transcription factor (Descombes & Schibler 1991). The truncated form of C/EBP$\alpha$ exhibits less efficient transcriptional activity than the full-length form (Ossipow *et al*. 1993). The truncated form also lacked antimitotic activity on 3T3-L1 preadipocytes (Lin *et al*. 1993), which is exhibited by the full-length form (Umek *et al*. 1991; Freytag & Geddes 1992).

Changes in the abundance and molar ratio of two C/EBP$\alpha$ forms were observed during liver development and during preadipocyte differentiation (Lin *et al*. 1993), while a report noted no apparent change in the ratio during liver development (Ossipow *et al*. 1993). The abundance and molar ratio of three C/EBP$\beta$ proteins also change during liver development (Descombes & Schibler 1991), in lipopolysaccharide-mediated acute-phase response (An *et al*. 1996), by intake of a low protein diet (Marten *et al*. 1996), and in the stimulation of human embryonal carcinoma cells by IL-6 and retinoic acid (Hsu & Chen-Kiang 1993).

Three protein species of C/EBP$\varepsilon$ are encoded by four mRNA isoforms generated by alternative promoter usage and differential splicing (Yamanaka *et al*. 1997b). The longest form transactivates the promoter of the granulocyte colony-stimulating factor receptor, while not the shortest form (Yamanaka *et al*. 1997b).

C/EBP$\gamma$ (Cooper *et al*. 1995) and CHOP/GADD153 (Ron & Habener 1992) have been interpreted mainly as negative transcription factors, while several recent reports describe transcriptional activation by CHOP/GADD153 (Ubeda *et al*. 1996; Wang & Ron 1996).

Chromosomal translocations yield fusion proteins of CHOP/GADD153 with FUS/TLS (*t*ranslocated in liposarcoma) (Crozat *et al*. 1993; Rabbitts *et al*. 1993) or EWS (*Ew*ing's *s*arcoma) (Panagopoulos *et al*. 1996, 1997) in human myxoid liposarcoma. Wild-type FUS/TLS and EWS are RNA-binding proteins (Crozat *et al*. 1993; Zinszner *et al*. 1994), and in fusion proteins the RNA-binding domain is replaced by the CHOP/GADD153 sequence. The fusion proteins exhibit oncogenic properties (Barone *et al*. 1994; Zinszner *et al*. 1994).

## Domain structures

### *bZIP domain*

The C/EBP family is characterized by the bZIP domain composed of two functional elements, that is, a basic DNA-binding region and a dimerization interface, the leucine zipper, located in relatively N-terminal and C-terminal sides, respectively (Landschulz *et al*. 1988b, 1989; Agre *et al*. 1989; Vinson *et al*. 1989).

*Basic region*. The basic region of bZIP interacts with the major groove of DNA in a sequence-specific manner (Nye & Graves 1990). As a consensus for high-affinity DNA sites recognized by C/EBP family members, a palindromic sequence ATTGCGCAAT was postulated (Vinson *et al*. 1989; Osada *et al*. 1996), while actual binding sites rather deviate from this consensus. Substitution of amino acid sequences within the basic region causes changes in DNA-binding specificity (Johnson 1993; Suckow *et al*. 1993; Koldin *et al*. 1995; Sera & Schultz 1996). Replacement of the basic region of C/EBP family members with other bZIP protein also leads to alteration of DNA-binding specificity for homodimers of or heterodimers containing resultant fusion proteins (Agre *et al*. 1989; Johnson 1993; Olive *et al*. 1996). DNA binding makes the basic region take $\alpha$-helical conformation in a induced fashion, prolonging the $\alpha$-helix of the leucine zipper domain (Shuman *et al*. 1990; O'Neil *et al*. 1991). When a designed acidic amphipathic sequence was introduced into the basic region, the resultant mutant protein exhibited the extended dimerization interface of the leucine zipper and brought about heterodimeric coiled-coil structure in combination with the basic region of the wild-type partner, preventing the basic region from DNA binding (Krylov *et al*. 1995).

*Leucine zipper and dimer formation*. The leucine zipper is an amphipathic $\alpha$-helix with a hydrophobic surface

containing repeated leucine residues at intervals of seven amino acids (Landschulz *et al*. 1988b, 1989). The hydrophobic surface serves as an interface of a bZIP dimer of a coiled-coil structure with each peptide arranged in a parallel orientation. Electrostatic interaction between charged amino acids flanking the hydrophobic surface contributes to determination of the dimerization specificity (Vinson *et al*. 1993).

C/EBP family members can homodimerize and heterodimerize with other members of the family (Landschulz *et al*. 1989; Descombes *et al*. 1990; Poli *et al*. 1990; Roman *et al*. 1990; Williams *et al*. 1991), and heterodimerize also with other bZIP protein family members producing complexes such as those between C/EBP$\beta$ and C/EBP-related activating transcription factor (C/ATF) (Vallejo *et al*. 1993), C/EBP$\beta$ and cAMP response element (CRE)-binding protein (CREB) (Tsukada *et al*. 1994), C/EBP$\beta$ and AP-1 family members Fos and Jun (Hsu *et al*. 1994) and C/EBP$\gamma$ and activating transcription factor 4 (ATF4) (Vinson *et al*. 1993). While it is not certain if CHOP/GADD153 can homodimerize or not (Ubeda *et al*. 1996), CHOP/GADD153 can heterodimerize with C/EBP$\alpha$ and C/EBP$\beta$ (Ron & Habener 1992; Fawcett *et al*. 1996; Ubeda *et al*. 1996). The resultant heterodimers do not bind to canonical C/EBP sites but do bind to deviated DNA sequences (Ubeda *et al*. 1996). CHOP/GADD153 also forms a heteromer with another stress-induced bZIP protein ATF3 and inhibits ATF3 binding to the ATF/CRE sites (Chen *et al*. 1996a). Generally, heterodimerization between proteins each having different DNA-binding specificity results in alteration of the target DNA sequence, and thus likely contributes to binding with DNA sequences considerably deviated from each of parental binding sequences. This may at least in part account for the extremely wide variety of target DNA sequences for protein complexes containing C/EBP family members.

The consequence of heterodimer formation can be both transcriptional activation and repression. C/EBP$\beta$ and C/EBP$\delta$ synergistically activate transcription from the IL-6 promoter in embryonic carcinoma cells (Kinoshita *et al*. 1992). The C/EBP$\beta$-C/ATF heterodimer cannot bind with a C/EBP$\beta$ site and this combination represses transcriptional activation that is caused by C/EBP$\beta$ through the C/EBP$\beta$ site in HepG2 cells, while the combination elicits synergistic transcriptional activation by binding to ceratin CRE sequences (Vallejo *et al*. 1993). The C/EBP$\beta$-C/ATF combination also elicits transcriptional activation through a pseudosymmetrical CRE of the PEPCK gene to the extent comparable to that by C/EBP$\beta$ alone (Vallejo *et al*. 1993). A similar pseudosymmetrical CRE-like sequence of the prointerleukin-1$\beta$ gene enhancer is recognized by the C/EBP$\beta$-CREB heterodimer and mediates activation by LPS and further augmentation by cAMP in human monocytic THP-1 cells (Tsukada *et al*. 1994). The C/EBP$\beta$-AP-1 heteromer cannot bind with a C/EBP$\beta$ site and represses transcriptional activation by C/EBP$\beta$ (Hsu *et al*. 1994). CHOP/GADD153 represses transcriptional activation of C/EBP$\alpha$ and C/EBP$\beta$ (Ron & Habener 1992; Fawcett *et al*. 1996; Ubeda *et al*. 1996) and also represses transcriptional inhibition of ATF3, by segregating these hetero-partners from their target sequences.

The hinge region (Johnson 1993) of 14-amino-acid residues between the leucine zipper and basic region seems to be involved in spacing of the half-site motif GCAAT in the dyad-symmetric target sequence ATTGCGCAAT.

### *Activation and inhibition domains*

The N-terminal portion of C/EBP$\alpha$ contains three separate domains that cooperatively activate transcription (Friedman & McKnight 1990; Pei & Shih 1991; Nerlov & Ziff 1994; Nerlov & Ziff 1995). Two N-terminal domains were shown to be required for binding with general transcription factors TBP and TFIIB (Nerlov & Ziff 1995). C/EBP$\beta$ also contains three N-terminal activation domains (Kowenz-Leutz *et al*. 1994; Trautwein *et al*. 1995; Williams *et al*. 1995), two of which locate in the regions homologous to those of C/EBP$\alpha$. C/EBP$\beta$ binds with a transcriptional coactivator p300 (Mink *et al*. 1997) through interaction between the E1 A-binding region of p300 and a wide range of amino-terminal activation domains of C/EBP$\beta$.

Interestingly, both C/EBP$\alpha$ (Pei & Shih 1991; Nerlov & Ziff 1994) and C/EBP$\beta$ (Kowenz-Leutz *et al*. 1994; Williams *et al*. 1995) bear negative regulatory or inhibitory domains, which mask activities of trans-activation domains (Pei & Shih 1991; Kowenz-Leutz *et al*. 1994; Nerlov & Ziff 1994; Williams *et al*. 1995) and the DNA-binding bZIP domain (Williams *et al*. 1995). These intramolecular inhibitions seem to be based on a tightly folded conformation which allows interactions between an inhibitory domain and a trans-activation domain or a DNA-binding domain (Kowenz-Leutz *et al*. 1994; Williams *et al*. 1995). As for C/EBP$\beta$, phosphorylation (Figure 1, Thr218 site of the chicken form) in an inhibitory domain is likely to result in liberation of trans-activation and DNA-binding domains from such a inhibitory interaction (Kowenz-Leutz *et al*. 1994). Another possible mechanism for relief of inhibition is interaction with other transcription factors as described below.

## Regulation by phosphorylation

Phosphorylation and dephosphorylation of C/EBP family members and their transcriptional consequences are summarized in Figure 1 and Table 1. Reports on regulation of C/EBP$\alpha$ by phosphorylation are limited. Dephosphorylation of C/EBP$\alpha$ by insulin in differentiated 3T3-L1 adipocytes was speculated to cause reduction of transcriptional activity and to result in decreased transcription of target genes such as the glucose transporter 4 gene (MacDougald *et al.* 1995; Hemati *et al.* 1997).

C/EBP$\beta$ is phosphorylated in a number of cells by various stimuli through pathways containing different kinases such as protein kinase A (PKA), protein kinase C (PKC), calcium calmodulin-dependent kinase (CaMK), and mitogen-activated protein (MAP) kinase (see Table 1). Generally, these phosphorylation events result in nuclear accumulation and/or transcriptional activation, while *in vitro* phosphorylation by PKA or PKC caused attenuation in DNA-binding activity (Trautwein *et al.* 1994). As described above, phosphorylation of chicken C/EBP$\beta$ Thr218 in the inhibition domain was postulated to cause abolishment of the repression effect (Kowenz-Leutz *et al.* 1994). This site is homologous to human C/EBP$\beta$ Thr235 that is the target of $p21^{ras}$-dependent MAP kinase (Nakajima *et al.* 1993).

Phosphorylation of C/EBP$\delta$ in hepatic cells in respose to inflammatory stimuli by turpentine (Ray & Ray 1994a; Ray & Ray 1994b), TNF$\alpha$ (Yin *et al.* 1996) and IL-1 (Lacorte *et al.* 1997), as well as *in vitro* phosphorylation by casein kinase II (Osada *et al.* 1996), caused increases in its DNA-binding activity or nuclear translocation. A report (Lacorte *et al.* 1997) noted that C/EBP$\delta$ phosphorylation is rather correlated with repression of the apolipoprotein C-III promoter.

CHOP/GADD153 undergoes phosphorylation by p38 MAP kinase in response to cellular stress such as treatment by an alkylating reagent methyl methanesulphonate, exhibiting increases in transcriptional activity and an inhibitory effect on adipose cell differentiation (Wang & Ron 1996). Fas-or $C_6$-ceramide-induced apoptosis in Jurkat T-lymphocytes was also correlated with phosphorylation of CHOP/GADD153 by JNK or p38 MAP kinase (Brenner *et al.* 1997).

Involvement of C/EBP family members in regulation of the gene for phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme of gluconeogenesis, in response to cAMP through the CRE (Park *et al.* 1990; Liu *et al.* 1991; Park *et al.* 1993; O'Brien *et al.* 1994) and/or other C/EBP sites (Roesler *et al.* 1995, 1996) has been repeatedly reported. Recent knockout studies (Croniger *et al.* 1997) noted that C/EBP$\alpha$ rather than C/EBP$\beta$ is more profoundly involved in cAMP response of the PEPCK gene, while the mechanism for the C/EBP$\alpha$-mediated cAMP response remains to be clarified.

## Interaction with other protein factors

Protein–protein interactions between C/EBP family members and a number of transcription factors of other classes, in addition to bZIP proteins described above, have been reported: C/EBP$\alpha$-NF-$\kappa$B (Vietor *et al.* 1996); C/EBP$\beta$-NF-$\kappa$B (LeClair *et al.* 1992; Matsusaka *et al.* 1993; Stein *et al.* 1993; Lee *et al.* 1996); C/EBP$\delta$-NF-$\kappa$B (Diehl & Hannink 1994; Ray *et al.* 1995); C/EBP$\beta$-glucocorticoid receptor (GR) (Nishio *et al.* 1993); C/EBP$\beta$-Sp1 (Lee *et al.* 1994, 1997b); C/EBP$\beta$-Myb (Mink *et al.* 1996); C/EBP$\alpha$-AML1 (Zhang *et al.* 1996). The leucine zipper of C/EBP$\beta$ and the Rel homology domain of NF-$\kappa$B (LeClair *et al.* 1992; Matsusaka *et al.* 1993; Stein *et al.* 1993; Lee *et al.* 1996) were shown to be interfaces of protein–protein association. These interfaces can be regarded as a contact point of two different signal transduction pathways each employing C/EBP$\beta$ and NF-$\kappa$B as a target in regulation of many immune response genes and acute-phase response genes. The amino-terminal 21 amino acid residues of the full-length form of C/EBP$\beta$ were proposed to be involved in functional synergism with NF-$\kappa$B (Lee *et al.* 1996). C/EBP$\beta$–GR association through the leucine zipper (Nishio *et al.* 1993) can be responsible for synergistic activation of the $\alpha_1$-acid glycoprotein gene by inflammatory cytokines and glucocorticoids in the acute-phase response.

Besides direct protein–protein interaction, a number of reports have been presented on functional interaction between C/EBP family members and other transcription factors. Liver-enriched members of the family C/EBP$\alpha$ and C/EBP$\beta$, in cooperation with other liver-selective transcription factors, activate target liver-specific genes, conferring a higher degree of specificity. C/EBP$\alpha$ and HNF-4 synergistically activate the apolipoprotein B promoter (Metzger *et al.* 1993). C/EBP$\alpha$ also activates the albumin promoter synergistically with HNF-1 (Wu *et al.* 1994). Combination of C/EBP$\beta$ and HNF-4 is essential for activation of the ornithine transcarbamylase enhancer (Nishiyori *et al.* 1994). Both C/EBP$\alpha$ and C/EBP$\beta$ can cooperate with HNF-1 in activation of the PEPCK promoter (Yanuka-Kashles *et al.* 1994).

Interaction of C/EBP$\beta$ with a ubiquitous factor Sp1 is required to promote C/EBP$\beta$ binding to a cryptic C/EBP site juxtaposed to an Sp1 site, in activation of the CYP2D5 P-450 gene in the liver during postnatal development (Lee *et al.* 1994, 1997b). Synergistic activation by C/EBP$\alpha$ in combination with another ubiquitous factor

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**Table 1**. Phosphorylation, dephosphorylation and related events of C/EBP family members

| Member | Species | Site and phosphorylation (↑) or dephosphorylation (↓) | Kinase and phosphatase | Stimulant, etc. | System mainly used | Effect | Reference |
|---|---|---|---|---|---|---|---|
| C/EBP$\alpha$ | Rat | Ser299↑ | PKC | — | *In vitro* | DNA binding↓ | Mahoney *et al.* (1992) |
| | Mouse | ?↓ | Protein phosphatase 1 or 2 A (through PI 3-kinase and FRAP) | Insulin | 3T3-L1 (mouse adipocytes) | Transcriptional activation↓? | MacDougald *et al.* (1995) Hemati *et al.* (1997) |
| C/EBP$\beta$ | Rat | ?↑ | ? | Forskolin | PC12 (rat pheochromocytoma) | Nuclear translocation↑ | Metz & Ziff (1991) |
| | Mouse | Ser276↑ | CaM kinase II | A23187 | G/C (rat pituitary tumour) | Transcriptional activation↑ | Wegner *et al.* (1992) |
| | Human | Thr235↑ | MAP kinase | $p21^{ras}$ transfection | 3T3 (mouse fibroblasts) transfection | Transcriptional activation↑ | Nakajima *et al.* (1993) |
| | Rat | Ser105↑ | ? (indirectly by PKC$\alpha$) | TPA | HepG2 (human hepatoma) transfection | Transcriptional activation↑ | Trautwein *et al.* (1993) |
| | Rat | Ser105↑ | PKA | — | *In vitro* | ? | Trautwein *et al.* (1994) |
| | | Ser173-Ser223, Ser240↑ | PKA | — | *In vitro* | DNA binding↓ | |
| | | Ser240↑ | PKC | — | *In vitro* | DNA binding↓ | |
| | Chicken | Thr218?↑ | MAP kinase? | *ts* v-*erb*B activation | HD3 (chicken erythroblast) | Transcriptional activation↑ | Kowenz-Leutz *et al.* (1994) |
| | Mouse | Ser No.?↑ | ? | cAMP | 30 A5 (mouse preadipocytes) | Transcriptional activation↑? | Tae *et al.* (1995) |
| | Rat | — | ? | TNF$\alpha$ | RALA255–10G (rat hepatocytes) | Nuclear translocation↑ | Yin *et al.* (1996) |
| | Rat | ?↑ | ? | Glutamate | Rat cortical astrocytes | ? | Yano *et al.* (1996) |
| | Human (rat?) | Ser299 (Ser299 of rat C/EBP$\alpha$? or Ser288 of human C/EBP$\beta$?)↑ | PKA | Pyrrolidinedithio-carbamate (antioxidant) | DKO-1 (human colorectal cancer) | Nuclear translocation↑ | Chinery *et al.* (1997a) |
| C/EBP$\delta$ | Rabbit | ?↑ | ? | Turpentine | Rabbit liver | DNA binding↑ | Ray & Ray (1994a) |
| | Mouse | ?↑ | ? | Okadaic acid, Vanadate (phosphatase inhibitors) | BNL CL.2 (mouse embryonic liver) transfection | | Ray & Ray (1994b) |
| | Rat | ?↑ | Casein kinase II | — | *In vitro* | DNA binding↑ | Osada *et al.* (1996) |
| | Rat | — | ? | TNF$\alpha$ | RALA255–10G (rat hepatocytes) | Nuclear translocation↑ | Yin *et al.* (1996) |
| | Human | Tyr No.?↑ | ? | IL-1 | HepG2 (human hepatoma) | DNA binding↑, Transcriptional activation↓? | Lacorte *et al.* (1997) |
| CHOP/ GADD153 | Mouse | Ser78, Ser81↑ | p38 MAP kinase | Methyl methanesulphonate | 3T3 (mouse fibroblasts) | Transcriptional activation↑ | Wang & Ron (1996) |
| | Human | ?↑ | JNK or p38 MAP kinase (through Rac) | Fas, $C_6$-Ceramide | Jurkat (human T cell leukaemia) | ? | Brenner *et al.* (1997) |

NF-Y (Milos & Zaret 1992) was implicated in dramatic activation of the albumin gene in the late foetal stage: NF-Y is likely to prepare an open chromatin configuration before the appearance of C/EBP family members.

p300 (Eckner *et al.* 1994) and its homologue the CREB-binding protein (CBP) (Chrivia *et al.* 1993) are transcriptional coactivators that relay the effects of many transcription factors to the basal transcriptional machinery via protein–protein interactions. A broad N-terminal region of chicken C/EBP$\beta$ was shown to interact with the E1 A-binding site of p300 (Mink *et al.* 1997). Combined with a report that a haematopoiesis-regulating transcription factor Myb interacts with the CREB-binding site of CBP (Dai *et al.* 1996; Oelgeschläger *et al.* 1996), it was proposed that p300/CBP mediates synergistic activation of *min-1*, a gene specifically expressed in the myelomonocytic lineage, by C/EBP$\beta$ and Myb (Burk *et al.* 1993; Ness *et al.* 1993). While C/EBP$\beta$ directly interacts with Myb and exhibits synergism (Mink *et al.* 1996), requirement of this interaction for the synergism seems to be overcome if p300/CBP is sufficiently available.

A tumour-suppressor protein the retinoblastoma protein (Rb) was shown to bind transiently with C/EBP$\beta$ in the course of differentiation of mouse fibroblasts into adipocytes, and to stimulate DNA binding and transactivation of C/EBP$\beta$ by a chaperone-like activity (Chen *et al.* 1996b). Analogous activation of C/EBP$\beta$ by Rb was also observed in monocyte/macrophage differentiation of human lymphoma cells (Chen *et al.* 1996c). C/EBP$\beta$ and other members of the family can be a target of one role of Rb, i.e. regulation of differentiation, which is distinguishable from another role, i.e. control of cell cycle.

C/EBP$\alpha$ binds and stabilizes p21, a cyclin-dependent kinase inhibitor (Timchenko *et al.* 1997). This interaction is likely to be responsible at least in part for C/EBP$\alpha$-mediated postnatal inhibition of hepatocyte proliferation. A similar mechanism seems to function also in growth arrest of preadipocytes (Timchenko *et al.* 1996). On the other hand, C/EBP$\alpha$ induced by glucocorticoids was postulated to be resposible for transcriptional activation of the gene for p21 in rat hepatoma cells (Cha *et al.* 1998; Cram *et al.* 1998), while in human colorectal cancer cells C/EBP$\beta$, but not C/EBP$\alpha$ nor C/EBP$\delta$, was shown to activate the p21 promoter (Chinery *et al.* 1997b).

## Regulation of genes for C/EBP family members

The C/EBP$\alpha$ gene is induced during terminal differentiation of cells such as hepatocytes (Birkenmeier *et al.* 1989) and adipocytes (Birkenmeier *et al.* 1989; Cao *et al.* 1991; Yeh *et al.* 1995). Once high-level expression is achieved, the C/EBP$\alpha$ gene in differentiated cells seems rather susceptible to repression by a number of stimuli (Table 2), while, in a rat hepatoma cell line, C/EBP$\alpha$ can be induced by dexamethasone and mediates the effects of the hormone causing $G_1$ cell cycle arrest (Ramos *et al.* 1996). During perinatal and neonatal periods, in the liver of hypothyroid rats, C/EBP$\alpha$ can be also induced by triiodothyronine and retinoic acid (Menéndez-Hurtado *et al.* 1997).

As shown in Figure 2, the mouse C/EBP$\alpha$ promoter is autoregulated directly by C/EBP$\alpha$ and/or a related factor(s) through binding to the promoter element in adipocytes (Christy *et al.* 1991) and liver (Legraverend *et al.* 1993). It was suggested that in preadipocytes, but not adipocytes, the autoactivation is prevented by a protein complex, which binds to the region overlapping to the C/EBP site, and which consists of Sp1, Sp1-like protein and AP-2$\alpha$ (Jiang *et al.* 1998), a factor previously designated C/EBP$\alpha$ undifferentiated protein (CUP) (Vasseur-Cognet & Lane 1993). The human C/EBP$\alpha$ promoter in hepatoma cells is indirectly autoregulated via stimulation by C/EBP$\alpha$ of binding of a ubiquitous factor named upstream stimulatory factor (USF) to the promoter element (Timchenko *et al.* 1995). These autoregulations may be involved in maintenance of high-level expression of the C/EBP$\alpha$ gene in differentiated cells.

The C/EBP$\beta$ gene is induced in many tissues and cells by various stimuli (Table 2). In the liver and hepatic cells, C/EBP$\beta$ mRNA levels are upregulated in the acute-phase response to inflammatory stimuli by LPS, IL-1, IL-6, IFN$\gamma$ and turpentine, as well as in response to hormones and related agents such as glucocorticoids, glucagon, cAMP, growth hormone, triiodothyronine and retinoic acid (Table 2). Response of the C/EBP$\beta$ gene to insulin is rather complicated. Insulin downregulates C/EBP$\beta$ gene expression in the mouse liver (Bosch *et al.* 1995), while, in a rat hepatoma cell line, insulin increases C/EBP$\beta$ mRNA and paradoxically represses transcription of the C/EBP$\beta$ gene stimulated by cytokines and dexamethasone (Campos & Baumann 1992). In 3T3-L1 differentiated adipocytes, insulin stimulates expression of the C/EBP$\beta$ gene (MacDougald *et al.* 1995).

Two CREB-binding sites were identified in the promoter region of the rat C/EBP$\beta$ gene (Figure 2), and shown to mediate the cAMP response of the gene via the PKA pathway in hepatoma and/or neuronal cells (Niehof *et al.* 1997). These sites can be targets for cAMP-inducing hormones such as glucagon, and for other such signals causing CREB phosphorylation via the MAP kinase pathway in the acute-phase response and liver regeneration. In transgenic mice, the 2.8-kb 5′-flanking region

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**Table 2.** Regulation of mRNA and protein levels for C/EBP family members

| Member | Species | System mainly used | Stimulant, etc. | Effect | Comment | Reference |
|---|---|---|---|---|---|---|
| C/EBP$\alpha$ | Mouse | 3T3-L1 (preadipocytes) | Dexamethasone + 3-isobutyl-1-methylxanthine (IBMX) (programmed administration) | mRNA ↑, Protein ↑ | Apparent at 3 days during differentiation | Cao *et al.* (1991)<br>Yeh *et al.* (1995) |
| | Mouse | Liver | LPS | Transcription ↓, mRNA ↓ | | Alam *et al.* (1992) |
| | | Lung, Fat tissue | LPS | mRNA ↓ | | |
| | Rat | Liver (regenerating) | Partial hepatectomy | Transcription ↓, mRNA ↓ | mRNA ↓ is prevented by cycloheximide (CHX) | Mischoulon *et al.* (1992) |
| | | Primary hepatocytes | EGF | mRNA ↓ | Prevented by CHX or puromycin (Pu), mRNA ↓ by actinomycin is also prevented by CHX or Pu | Rana *et al.* (1995) |
| | Mouse | 32D C13 (myelomonoblastic cells) | G-CSF (withdrawal of IL-3) | Protein ↑ | Protein ↓ afterward during differentiation | Scott *et al.* (1992) |
| | Rat | Liver (regenerating) | Partial hepatectomy | Transcription ↓, mRNA ↓, Protein ↓ | | Flodby *et al.* (1993) |
| | Rat | Liver | cAMP | mRNA ↓ | | Park *et al.* (1993) |
| | Rat | Ovary granulosa cells | Human chorionic gonadotropin | mRNA ↓ | | Sirois & Richards (1993) |
| | Rat | H4IIE (hepatoma) | Amino acid limitation | mRNA ↑ | | Marten *et al.* (1994) |
| | Mouse | 3T3-L1 (differentiated adipocytes) | Dexamethasone, Triaminolone acetonide | Transcription ↓, mRNA ↓, Protein ↓ | | MacDougald *et al.* (1994) |
| | Rat | White adipose tissue | | Protein ↓ | | |
| | Rabbit | Liver | Turpentine | Protein ↓<br>mRNA ↓ | | Ray & Ray (1994a)<br>Ray & Ray (1994b) |
| | Mouse | 3T3-L1 (differentiated adipocytes) | Insulin | Transcription ↓, mRNA ↓, Protein ↓ | | MacDougald *et al.* (1995) |
| | Mouse | 30 A5 (preadipocytes) | cAMP + IBMX | mRNA ↑ | Repressed by TNF$\alpha$ | Tae *et al.* (1995) |
| | Rat | Liver (carcinogenic) | Diethylnitrosamine, etc. | Protein ↓ | | Osada *et al.* (1995) |
| | Rat | BDS1 (hepatoma) | Dexamethasone | mRNA ↑, Protein ↑ | | Ramos *et al.* (1996) |
| | Rat | Liver (perinatal and neonatal hypothyroid animals) | Triiodothyronine (T3)<br>Retinoic acid (RA) | mRNA ↑, Protein ↑<br>mRNA ↑ | Protein induced faster than mRNA<br>RA is more potent than T3 | Menéndez-Hurtado *et al.* (1997) |
| C/EBP$\beta$ | Mouse | Liver and other organs | LPS, IL-1, IL-6 | mRNA ↑ | | Akira *et al.* (1990)<br>Isshiki *et al.* (1991) |
| | Mouse | 3T3-L1 (preadipocytes) | IBMX | mRNA ↑, Protein ↑ | | Cao *et al.* (1991)<br>Yeh *et al.* (1995) |
| | Mouse | M1 (myeloid leukaemia) | IL-6 | mRNA ↑ | | Natsuka *et al.* (1992) |
| | Human | U937 (histiocytic leukaemia) | LPS, PMA | mRNA ↑ | | |
| | | HL-60 (promyelocytic leukaemia) | PMA | mRNA ↑ | | |
| | | Cultured peripheral monocytes | | LPS | mRNA ↑ | |
| | Mouse | Liver and other organs | LPS | mRNA ↑ | | Alam *et al.* (1992) |
| | | Kidney | LPS | Transcription ↑, mRNA ↑ | | |
| | Rat | H-35 (hepatoma) | Insulin | Transcription ↓, mRNA ↑ | Paradoxical effects on transcription and mRNA levels | Campos & Baumann (1992) |
| | Rat | H-35 (hepatoma) | IL-1+IL-6+ Dexamethasone<br>Dexamethasone | Transcription ↑, mRNA ↑, Protein ↑<br>mRNA ↑ | | Baumann *et al.* (1992) |
| | Mouse | 32D C13 (myelomonoblastic cells) | G-CSF (withdrawal of IL-3) | Protein ↑ | | Scott *et al.* (1992) |

**Table 2.** Continued

| Member | Species | System mainly used | Stimulant, etc. | Effect | Comment | Reference |
|---|---|---|---|---|---|---|
| | Rat | Liver (regenerating) | Partial hepatectomy | Transcription ↑, mRNA ↑, Protein ↑ | Induced also after sham operation | Flodby *et al.* (1993) |
| | Rat | Primary hepatocytes | Growth hormone (4 days) | mRNA ↑ | | Potter *et al.* (1993) |
| | Rat | Liver | cAMP | mRNA ↑ | | Park *et al.* (1993) |
| | Rat | Ovary granulosa cells | Human chorionic gonadotropin | mRNA ↑, Protein ↑ | | Sirois & Richards (1993) |
| | Rat | H4IIE (hepatoma) | Amino acid limitation | mRNA ↑ | | Marten *et al.* (1994) |
| | Rat | Liver | LPS | mRNA ↑ | | Sylvester *et al.* (1994) |
| | Rabbit | Liver | Turpentine | mRNA ↑ | | Ray & Ray (1994b) |
| | Mouse | 3T3-L1 (differentiated adipocytes) | Insulin | Transcription ↑, mRNA ↑, Protein ↑ | | MacDougald *et al.* (1995) |
| | Mouse | Liver | High carbohydrate diet, Insulin | mRNA ↓, Protein ↓ | | Bosch *et al.* (1995) |
| | Mouse | 30 A5 (preadipocytes) | cAMP + IBMX | mRNA ↑, Protein ↑ | | Tae *et al.* (1995) |
| | Mouse | 3T3-*F*442 A (preadipocytes) | Growth hormone | Protein ↑ | No change in mRNA levels | Clarkson *et al.* (1995) |
| | Mouse | Cultured cortical astrocytes | Vasoactive intestinal peptide, Pituitary adenylate cyclase-activating peptide, Noradrenaline, cAMP, Forskolin | mRNA ↑, Protein ↑ | | Cardinaux & Magistretti (1996) |
| | Rat | PC12 (pheochromocytoma) | Arsenite | mRNA ↑, Protein ↑ | | Fawcett *et al.* (1996) |
| | Rat | Primary hepatocytes | Dexamethasone, Glucagon | Transcription ↑, mRNA ↑ | | Matsuno *et al.* (1996) |
| | Rat | Cultured cortical astrocytes | Glutamate | mRNA ↑, Protein ↑ | | Yano *et al.* (1996) |
| | Rat | Liver (regenerating) | Partial hepatectomy | mRNA ↑, Protein ↑ | | Trautwein *et al.* (1996) |
| | Rat | H4IIE (hepatoma) | Dexamethasone | mRNA ↑ | | Gotoh *et al.* (1997) |
| | Rat | Cultured peritoneal macrophage | LPS | mRNA ↑ | Sonoki *et al.* (1997) | |
| | Rat | Ovary granulosa cells Ovary theca cells | Human chorionic gonadotropin | Protein ↑ | | Pall *et al.* (1997) |
| | Rat | Liver (perinatal and neonatal hypothyroid animals) | Triiodothyronine (T3) | mRNA ↑, Protein ↑ | Protein induced faster than mRNA | Menéndez-Hurtado *et al.* (1997) |
| | | | Retinoic acid (RA) | mRNA ↑ | RA is more potent than T3 | |
| C/EBP$\delta$ | Mouse | 3T3-L1 (preadipocytes) | Dexamethasone | mRNA ↑, Protein ↑ | | Cao *et al.* (1991) Yeh *et al.* (1995) |
| | Mouse | Liver and other organs | LPS | mRNA ↑ | | Kinoshita *et al.* (1992) |
| | Mouse | Liver and other organs | LPS | mRNA ↑ | | Alam *et al.* (1992) |
| | | Kidney | LPS | Transcription ↑, mRNA ↑ | | |
| | Mouse | 32D C13 (myelomonoblastic cells) | G-CSF (withdrawal of IL-3) | Protein ↑ | Protein ↓ afterward during differentiation | Scott *et al.* (1992) |
| | Rat | Liver (regenerating) | Partial hepatectomy | Transcription ↑, mRNA ↑, Protein ↑ | | Flodby *et al.* (1993) |
| | Human | Hep3B (hepatoma) | IL-6 | mRNA ↑, Protein ↑ | | Ramji *et al.* (1993) |
| | Human | Hep3B2 (hepatoma) | IL-1 | mRNA ↑, Protein ↑ | | Juan *et al.* (1993) |
| | Rat | Liver | LPS | mRNA ↑ | | Sylvester *et al.* (1994) |
| | Mouse | 3T3-L1 (differentiated adipocytes) | Dexamethasone, Triaminolone acetonide | Transcription ↑, mRNA ↑, Protein ↑ | | MacDougald *et al.* (1994) |
| | Rat | White adipose tissue | | Protein ↑ | | |
| | Rabbit | Liver | Turpentine | Protein ↑ | | Ray & Ray (1994a) |
| | | | | mRNA ↑ | | Ray & Ray (1994b) |

 

**Table 2.** Continued

| Member | Species | System mainly used | Stimulant, etc. | Effect | Comment | Reference |
|---|---|---|---|---|---|---|
| | Mouse | 3T3-L1 (differentiated adipocytes) | Insulin | Transcription ↑, mRNA ↑, Protein ↑ | | MacDougald *et al.* (1995) |
| | Mouse | 3T3-*F*442 A (preadipocytes) | Growth hormone | Transcription ↑, mRNA ↑, Protein ↑ | | Clarkson *et al.* (1995) |
| | Mouse | Cultured cortical astrocytes | Vasoactive intestinal peptide, Pituitary adenylate cyclase-activating peptide, Noradrenaline, cAMP, Forskolin | mRNA ↑, Protein ↑ | | Cardinaux & Magistretti (1996) |
| | Rat | Cultured cortical astrocytes | Glutamate | mRNA ↓, Protein ↓ | | Yano *et al.* (1996) |
| C/EBPε | Human | HL-60 (promyelocytic leukaemia) | Retinoic acid | mRNA ↑ | | Yamanaka *et al.* (1997b) |
| CHOP/GADD153 | Human | HeLa (cervical cancer) | Methyl methanesulphonate(MMS) | mRNA ↑ | | Fornace *et al.* (1989) |
| | | | Medium depletion | mRNA ↑ | | |
| | | HL-60 (promyelocytic leukaemia) | MMS | mRNA ↑ | | |
| | | Cultured skin fibroblasts | MMS, Contact inhibition | mRNA ↑ | | |
| | Chinese hamster | CHO (ovary cells) | MMS, Medium depletion, Reduced serum, Hydroxyurea | mRNA ↑ | | |
| | | V79 (lung fibroblasts) | MMS | mRNA ↑ | | |
| | Mouse | 3T3 (embryonic fibroblasts) | Contact inhibition | mRNA ↑ | | |
| | | Hepa-1 (hepatoma) | MMS | mRNA ↑ | | |
| | Pig | LLC-PK1 (renal epithelial cells) | Cysteine conjugates | mRNA ↑ | Partially prevented by cycloheximide (CHX) | Chen *et al.* (1992) |
| | | | DTT | mRNA ↑ | | |
| | Human | HeLa (cervical cancer) | A23187 ($Ca^{2+}$ ionophore) | Transcription ↑, mRNA ↑, mRNA stability ↑ | Prevented by BAPTA-AM and EGTA that also block effects of MMS | Bartlett *et al.* (1992) |
| | Mouse | 3T3 (embryonic fibroblasts) | Thapsigargin | mRNA ↑ | Prevented by H7 or 2-aminopurine but not by genistein nor CHX | Price & Calderwood (1992) |
| | | | Tunicamycin, A23187, | mRNA ↑ | | |
| | | | Hypoxia | mRNA ↑ | Not prevented by genistein nor CHX | |
| | Mouse | 3T3-L1 (preadipocytes) | Differentiation to adipocyte | mRNA ↑ | | Ron & Habener (1992) |
| | | | MMS | Protein ↑ | | |
| | | 3T3-L1 (differentiated adipocytes) | Dedifferentiation | Protein ↓ | | |
| | Human | HeLa (cervical cancer) | Glucose deprivation | Transcription ↑, mRNA ↑, Protein ↑ | | Carlson *et al.* (1993) |
| | Mouse | 3T3-L1 (preadipocytes) | Glucose deprivation | mRNA ↑ | | |
| | | 3T3-L1 (differentiated adipocytes) | Glucose deprivation | mRNA ↑ | | |
| | | 3T3-L1 (preadipocytes) | Differentiation to adipocytes | mRNA ↑ | Prevented by glucose addition | |
| | Rat | Liver | LPS | mRNA ↑ | Presumably mediated by C/EBPβ | Sylvester *et al.* (1994) |
| | Rat | H4IIE (hepatoma) | Amino acid limitation | mRNA ↑ | | Marten *et al.* (1994) |
| | Mouse | 3T3-L1 (preadipocytes) | Low glucose concentration during adipocyte differentiation | Protein ↑ | | Batchvarova *et al.* (1995) |
| | Human | HeLa (cervical cancer) | $H_2O_2$ and other free-radical generators | mRNA ↑, Promoter activity ↑ | Potentiated by buthionine sulphoximine, Prevented by *N*-acetyl-cysteine, Effects of $H_2O_2$, but not of arsenite, is prevented by *o*-phenanthroline or mannitol | Guyton *et al.* (1996) |
| | | WI38 (lung cells) | Arsenite and other thiol-reactive reagents | mRNA ↑ | | |
| | Mouse | 3T3 (embryonic fibroblasts) | MMS, Tunicamycin | Protein ↑ | | Ubeda *et al.* (1996) |

 

**Table 2.** Continued

| Member | Species | System mainly used | Stimulant, etc. | Effect | Comment | Reference |
|---|---|---|---|---|---|---|
| | Rat | Liver | $CCl_4$, Partial hepatectomy | mRNA ↓ | | Chen *et al.* (1996) |
| | Rat | PC12 (pheochromocytoma) | Arsenite | mRNA ↑, Protein ↑ | | Fawcett *et al.* (1996) |
| | Mouse | 3T3 (embryonic fibroblasts) | MMS, Tunicamycin, Low glucose concentration, Dinitrophenol | mRNA ↑ | CHOP induction by MMS or tunicamycin in CHO cells is attenuated by overexpression of an endoplasmic reticulum chaperon BiP | Wang *et al.* (1996) |
| | Chinese hamster | CHO K12 (ovary cells, *ts* mutant of N-linked glycosylation) | Shift to nonpermissive temperature | Protein ↑ | | |
| | Syrian hamster | BHK tsBN7 (kidney, *ts* N-linked glycosylation) | Shift to nonpermissive temperature | Protein ↑ | | |
| | Human | HeLa (cervical cancer) | Leucine starvation | Transcription ↑, mRNA ↑, mRNA stability ↑, Protein ↑ | mRNA ↑ is dependent on *de novo* protein synthesis | Bruhat *et al.* (1997) |
| | Human | HepG2 (hepatoma) | Leucine starvation | mRNA ↑ | | |
| | Human | Caco-2 (colon carcinoma) | Leucine starvation | mRNA ↑ | | |

of the rat C/EBP$\beta$ gene directs high-level, position-independent, copy number-dependent expression, resembling the locus control region (Talbot *et al.* 1994).

The C/EBP$\delta$ gene is also induced by LPS, IL-1, IL-6, IFN$\gamma$ and turpentine in the liver and other organs and hepatoma cell lines (Table 2), the extent of the induction being more dramatic than that of the C/EBP$\beta$ gene (Alam *et al.* 1992; Sylvester *et al.* 1994; Cantwell *et al.* 1998). In the promoter region of the mouse C/EBP$\delta$ gene (Figure 2), a DNA element was identified that binds with a factor named signal transducer and activator of transcription (Stat) 3 and that mediates IL-6 resposiveness in hepatoma cells (Cantwell *et al.* 1998). In preadipocytes and/or adipocytes, the C/EBP$\delta$ gene is induced in respose to hormones such as glucocorticoids, insulin and growth hormone (Table 2).

High expression of the human C/EBP$\varepsilon$ gene is detected in tissues including peripheral blood leucocytes, bone marrow and ovary, and cell lines such as promyelocytic leukaemia HL60 (Antonson *et al.* 1996; Chumakov *et al.* 1997). The C/EBP$\varepsilon$ gene is induced during granulocyte differentiation *in vitro* and treatment of HL60 cells by retinoic acid (Yamanaka *et al.* 1997b).

As seen from the fact that the CHOP/GADD153 gene was first characterized as the growth arrest- and DNA damage-inducible gene (Fornace *et al.* 1989), this gene is induced in response to various cellular stresses including nutrient deprivation, UV irradiation, and exposure to genotoxic reagents, cysteine conjugates, DTT, calcium ionophore, LPS, arsenite, oxidants and tunicamycin (Table 2). It was remarked (Chen *et al.* 1992; Price & Calderwood 1992; Wang *et al.* 1996) that some of these stimuli cause also perturbation of protein folding especially in the endoplasmic reticulum (ER), as was typically exemplified by tunicamycin that is an inhibitor of protein glycosylation. The ER stresses strongly induce the CHOP/GADD153 gene (Price & Calderwood 1992; Wang *et al.* 1996). Overexpression of the CHOP/GADD153 gene (Fornace *et al.* 1989; Kelsey *et al.* 1993) in the liver of $c^{14CoS}$ mice that are deficient in the fumarylacetoacetate hydrolase gene was corrected by transgenic rescue of the hydrolase gene (Kelsey *et al.* 1993).

The hamster CHOP/GADD153 promoter (Figure 2) contains a C/EBP-binding site (Sylvester *et al.* 1994; Fawcett *et al.* 1996) and an AP-1-binding site (Guyton *et al.* 1996). C/EBP$\beta$ induced by LPS in the liver (Sylvester *et al.* 1994) and by arsenite in rat pheochromocytoma PC12 cells (Fawcett *et al.* 1996) is likely to stimulate the CHOP/GADD153 promoter via binding to the C/EBP site. Induced CHOP/GADD153 may in turn heterodimerize with C/EBP$\beta$ and repress its own gene by sequestrating C/EBP$\beta$ from the binding site (Fawcett *et al.* 1996).

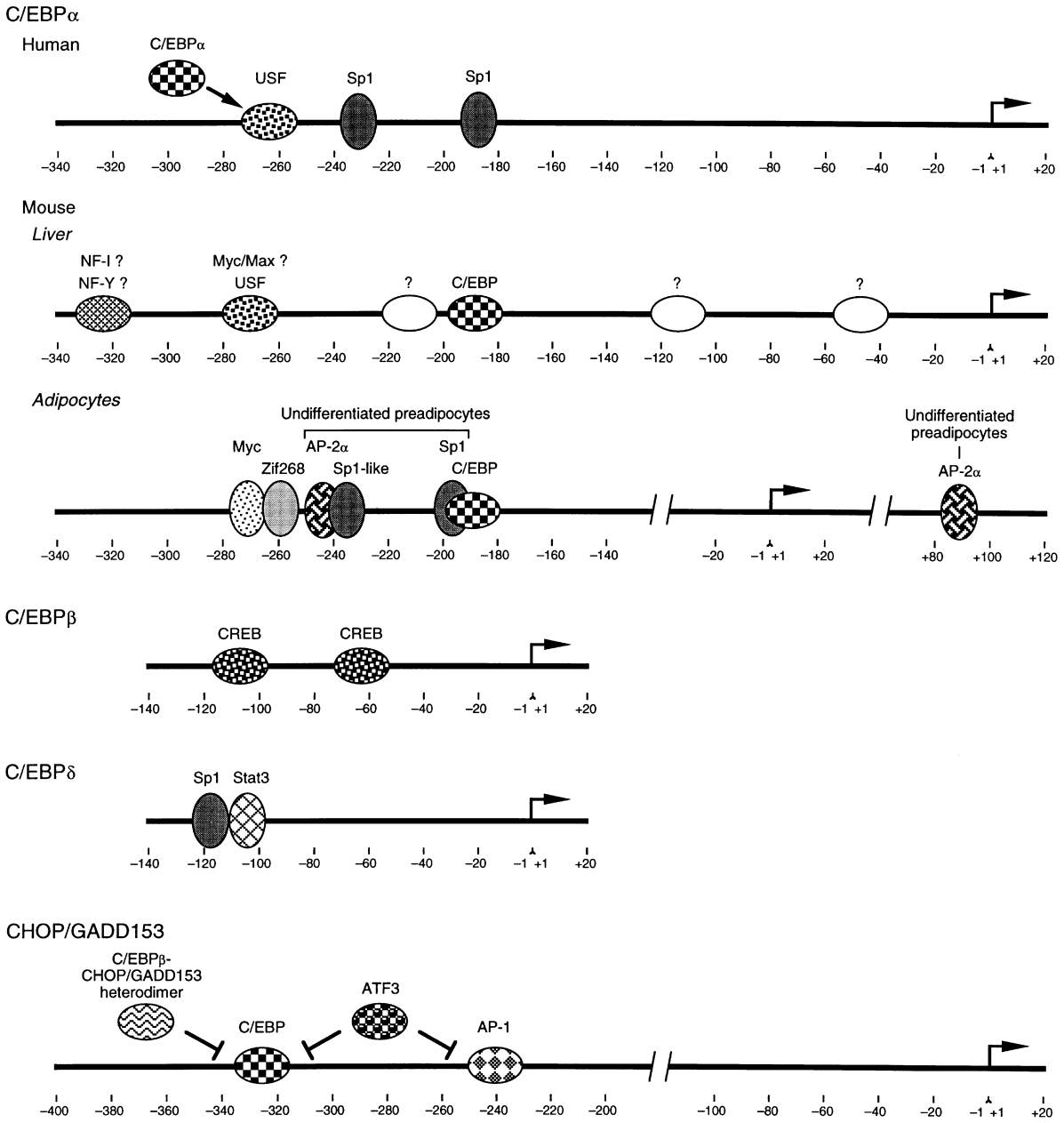


**Figure 2.** Factors interacting with regulatory elements of genes for C/EBP family members. See the text for explanation. References are as follows. C/EBP$\alpha$: human (Timchenko *et al.* 1995); mouse liver (Legraverend *et al.* 1993); mouse adipocytes (Christy *et al.* 1991; Vasseur-Cognet & Lane 1993; Tang *et al.* 1997; Jiang *et al.* 1998). C/EBP$\beta$: (Niehof *et al.* 1997). C/EBP$\delta$: (Cantwell *et al.* 1998). CHOP/GADD153: (Sylvester *et al.* 1994; Fawcett *et al.* 1996; Guyton *et al.* 1996; Wolfgang *et al.* 1997).

Exposure of HeLa cells to oxidants or UV irradiation stimulates binding of Fos and Jun to the AP-1 site that is important in transcriptional activation of the CHOP/GADD153 gene by these stimuli (Guyton *et al.* 1996). Both C/EBP site and AP-1 site are bound also by another stress-inducible gene product ATF3, and mediate inhibition of the CHOP/GADD153 promoter by this factor (Wolfgang *et al.* 1997). ATF3 induced in the liver of $CCl_4$-treated rats (Wolfgang *et al.* 1997) can be responsible for repression of the the CHOP/GADD153 gene by $CCl_4$ (Chen *et al.* 1996a). Combined with attenuation of trans-repressing activity of ATF3 by CHOP/GADD153 (Chen *et al.* 1996a), CHOP/GADD153 and ATF3 are mutually negative regulators.

## Clarified and newly proposed questions by gene knockout studies

Targeted disruption of the C/EBP$\alpha$ gene in mice caused neonatal death due to hypoglycemia associated with impaired expression of the gluconeogenic enzymes such as PEPCK and glucose-6-phosphatase in the liver (Wang *et al*. 1995; Flodby *et al*. 1996). Decreases in mRNA levels for glycogen synthase (Wang *et al*. 1995), serun albumin (Wang *et al*. 1995; Flodby *et al*. 1996) and ornithine cycle enzymes (Kimura *et al*. 1998) were also observed. A conditional knockout study using targeted insertion of *loxP* sequences followed by delivery of a Cre recombinase adenovirus preferentially into the liver showed that the adult C/EBP$\alpha$ deficiency results in severe jaundice associated with reduced expression of bilirubin UDP-glucuronosyltransferase (Lee *et al*. 1997a). In the liver of this conditional knockout mouse, decreases in mRNA levels for blood coagulation factor IX were also detected (Lee *et al*. 1997a). Therefore, C/EBP$\alpha$-deficient mice exhibit disorders of expression of a number of liver-specific genes. These results are concordant with observations for interaction of C/EBP$\alpha$ and other C/EBP family members with regulatory regions of genes for PEPCK (Park *et al*. 1990, 1993; O'Brien *et al*. 1994; Yanuka-Kashles *et al*. 1994; Liu & Curthoys 1996; Nizielski *et al*. 1996; Roesler *et al*. 1996), serum albumin (Costa *et al*. 1988; Friedman *et al*. 1989; Herbst *et al*. 1989; Maire *et al*. 1989; Milos & Zaret 1992; Nerlov & Ziff 1994; Trautwein *et al*. 1996), factor IX (Crossley & Brownlee 1990; Picketts *et al*. 1993) and ornithine cycle enzymes (Howell *et al*. 1989; Murakami *et al*. 1990; Takiguchi & Mori 1991; Kimura *et al*. 1993; Gotoh *et al*. 1994; Nishiyori *et al*. 1994; Chowdhury *et al*. 1996; Gotoh *et al*. 1997; reviewed in Takiguchi & Mori 1995).

Increases in proliferating hepatocytes were detected in the C/EBP$\alpha$-deficient liver, with elevation in BrdU uptake, proliferating cell nuclear antigen (PCNA) immunostaining, and mRNA levels of Myc and Jun (Flodby *et al*. 1996; Timchenko *et al*. 1997). This is consistent with the anti-proliferating role of C/EBP$\alpha$ revealed by transfection experiments using adipocytes (Umek *et al*. 1991), hepatic cells (Hendricks-Taylor & Darlington 1995; Diehl *et al*. 1996; Ramos *et al*. 1996) and other cells (Hendricks-Taylor & Darlington 1995; Timchenko *et al*. 1996). Correlation between hepatocyte proliferation and repression of the C/EBP$\alpha$ gene was repeatedly noted in regenerating liver (Mischoulon *et al*. 1992; Flodby *et al*. 1993; Rana *et al*. 1995; Trautwein *et al*. 1996) and primary hepatocyte culture (Mischoulon *et al*. 1992; Rana *et al*. 1994, 1995). Growth-inhibitory roles of C/EBP$\beta$ in hepatoma cells (Buck *et al*. 1994) and of CHOP/GADD153 in fibroblasts (Barone *et al*. 1994) were also reported. As described above, stabilization of p21 by C/EBP$\alpha$ through protein–protein interaction (Timchenko *et al*. 1997) and activation of the p21 gene by C/EBP$\alpha$ (Cram *et al*. 1998) were postulated as mechanisms of C/EBP$\alpha$-mediated growth arrest.

In C/EBP$\alpha$-null mice, lack of lipid accumulation in adipose tissue (Wang *et al*. 1995), hyperproliferation of type II pneumocytes in the lung (Flodby *et al*. 1996) and absence of neutrophils and eosinophils associated with loss of the granulocyte colony-stimulating factor receptor (Zhang *et al*. 1997) were also observed. The crucial role of C/EBP$\alpha$ in adipogenesis of cell lines has been repeatedly noted (Christy *et al*. 1989; Samuelsson *et al*. 1991; Umek *et al*. 1991; Lin & Lane 1992; Freytag *et al*. 1994).

C/EBP$\beta$-deficiency caused impairment of macrophage bactericidal and tumoricidal activities (Tanaka *et al*. 1995), and a lymphoproliferative disorder with distorted humoral, innate and cellular immunity (Screpanti *et al*. 1995). Induction of the granulocyte colony-stimulating factor gene in macrophages and fibroblasts (Tanaka *et al*. 1997) and increases in the serum IL-12 levels during the course of delayed-type hypersensitivity (Screpanti *et al*. 1995) are reduced in C/EBP$\beta$-deficient mice.

Female C/EBP$\beta$-deficient mice are infertile, and lack of corpora lutea resulting from defective granulosa cell function at the postovulatory stage explained this infertility (Sterneck *et al*. 1997). Downregulation of mRNA levels for prostaglandin endoperoxide synthase 2 and P450 aromatase in response to gonadotropins are lost in the C/EBP$\beta$-deficient ovary (Sterneck *et al*. 1997). A study using *ex vivo* perfusion of rat ovary showed that treatment with antisense oligonucleotides against C/EBP$\beta$ inhibits ovulation in response to luteinizing hormone (Pall *et al*. 1997).

Differentiation of brown adipose tissue of the interscapular region is moderately disordered in C/EBP$\beta$-deficient mice, and more severely in C/EBP$\beta$ · C/EBP$\delta$ double knockout mice (Tanaka *et al*. 1997). Weight of epididymal white adipose tissue is significantly decreased in the double knockout mice. *In vitro* differentiation of embryonic fibroblasts into adipocytes is also reduced moderately in C/EBP$\beta$-deficieny, and more profoundly in the double knockout. Studies on adipogenic differentiation of cell lines led to the proposal for the sequential gene cascade: C/EBP$\beta$ and C/EBP$\delta$ synergistically induce genes for C/EBP$\alpha$ and PPAR$\gamma$, which then cooperatively activate adipogenic genes (Hu *et al*. 1995; Wu *et al*. 1995, 1996; Yeh *et al*. 1995; Schwarz *et al*. 1997). Concordant with this proposal, expression of genes for C/EBP$\alpha$ and PPAR$\gamma$ is severely impaired during *in vitro* differentiation of the double knockout

embryonic fibroblasts (Tanaka *et al*. 1997). However, *in vivo*, normal expression of C/EBP$\alpha$ and PPAR$\gamma$ genes was observed in C/EBP$\beta$·C/EBP$\delta$-deficient adipose tissues, despite impaired adipogenesis. This suggests the presence of an alternative gene cascade *in vivo* for activation of adipogenic genes under the control of C/EBP$\beta$ and C/EBP$\delta$.

As for the liver-selective gene, expression of the P450 gene *CYP2D11* was shown to be severely reduced in C/EBP$\beta$-null mice (Lee *et al*. 1997b), consistent with activation of the promoter of this gene specifically by C/EBP$\beta$ (Lee *et al*. 1994, 1997b).

It has been repeatedly postulated that C/EBP$\beta$ and C/EBP$\delta$ can mediate the acute-phase response through binding to IL-6-responsive elements of target genes, in addition to Stat3 that binds to another class of IL-6-responsive elements, and NF-$\kappa$B that mediates the effects of IL-1 (as a recent review, see introduction of Cantwell *et al*. 1998). As mentioned above, phosphorylation of C/EBP$\beta$ and subsequent nuclear accumulation and/or stimulation of transactivator activity, as well as inductiuon of the gene for C/EBP$\delta$, are likely to be major mechanistic causes for activation of downstream target genes in the acute-phase response. It was briefly noted that induction of acute-phase response genes was moderately impaired in C/EBP$\beta$-deficient liver (Screpanti *et al*. 1995). Possible involvement of C/EBP$\beta$ and C/EBP$\delta$ in hormonal regulation (see Table 2) and liver regeneration (Flodby *et al*. 1993; Rana *et al*. 1995; Trautwein *et al*. 1996; Jiang & Zarnegar 1997) have been also repeatedly noted. Availability of C/EBP$\beta$-, C/EBP$\delta$-, and double-knockout mice provides an opportunity to test these hypotheses.

C/EBP$\varepsilon$-deficient mice fail to generate mature granulocytes and cause myelodysplasia (Yamanaka *et al*. 1997a). Opportunistic infections resulted in early lethality. While mRNA levels for receptors of colony-stimulating factors are elevated in the bone marrow of C/EBP$\varepsilon$-deficient mice, mRNA levels for IFN$\gamma$, IL-2, IL-4, IL-12p40 and TNF$\alpha$ are decreased.

Embryonic fibroblasts derived from CHOP/GADD153-deficient mice exhibit increased resistance to programmed cell death in response to ER stress (Zinszner *et al*. 1998). A similar resistance was also observed in embryonic fibroblasts deficient in C/EBP$\beta$, a major dimerization partner of CHOP/GADD153. Overexpressed chicken C/EBP$\beta$ was shown to induce apoptosis of myeloid/erythroid progenitor cells (Müller *et al*. 1995). *In vivo*, tunicamycin-treated CHOP/GADD153-deficient mice display decreased programmed cell death and less evident subsequent cellular regeneration of the renal proximal tubular epithelium (Zinszner *et al*. 1998). During apoptosis of human leukaemic Jurkat cells in response to stimulation of the receptor Fas with the anti-Fas antibody, CHOP/GADD153 is strongly phosphorylated via the JNK or p38 MAP kinase pathway (Brenner *et al*. 1997). Intraperitoneal administration of the anti-Fas antibody into mice causes severe damage of the liver, resembling fulminant hepatitis (Ogasawara *et al*. 1993). The anti-Fas antibody also induces the programmed cell death of primary-cultured mouse hepatocytes (Ni *et al*. 1994). Investigation on the possible role of CHOP/GADD153 and other C/EBP family members in apoptosis of hepatocytes would be interesting.

## Acknowledgements

I thank M. Mori and S. Fujimura for support and encouragement. I am also grateful to A. Nishiyori, A. Kimura, T. Gotoh, H. Tashiro, F. Matsuno, S. Chowdhury, K. Iwase, T. Kimura and other colleagues for discussions and suggestions. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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