Biological Role of the CCAAT/Enhancer-Binding Protein
Family of Transcription Factors*

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CCAT/enhancer-binding proteins (C/EBPs) comprise a family of transcription factors that are critical for normal cellular differentiation and function in a variety of tissues. The prototypic C/EBP is a modular protein, consisting of an activation domain, a dimerization bZIP region, and a DNA-binding domain. All family members share the highly conserved dimerization domain, required for DNA binding, by which they form homo- and heterodimers with other family members. C/EBPs are least conserved in their activation domains and vary from strong activators to dominant negative repressors. The pleiotropic effects of C/EBPs are in part because of tissue- and stage-specific expression. Dimerization of different C/EBP proteins precisely modulates transcriptional activity of target genes. Recent work with mice deficient in specific C/EBPs underscores the effects of these factors in tissue development, function, and response to injury.

The CCAAT/enhancer-binding proteins (C/EBPs) encompass a family of transcription factors with structural as well as functional homologies. Similarities between C/EBP family members suggest an evolutionary history of genetic duplications with subsequent pressure to diversify. The resulting family of proteins varies in tissue specificity and transactivating ability. Since the cloning of the family's original member, C/EBPα, nearly a decade ago, five other C/EBPs have been identified that interact with each other and transcription factors in other protein families to regulate mRNA transcription. The pleiotropic effects of C/EBPs are in part because of tissue- and stage-specific expression, leaky ribosomal reading, post-transcriptional modifications, and variable DNA binding specificities. These mechanisms result in variable amounts of the C/EBP isoforms, available to dimerize and bind to cognate sites in different tissues. Recent work with mice genetically altered to abolish expression of C/EBPs underscores the role these factors play in normal tissue development and cellular function, cellular proliferation, and functional differentiation.

The prototypic C/EBP, like many transcription factors, is a modular protein, consisting of an activation domain, a DNA-binding basic region, and a leucine-rich dimerization domain. The dimerization domain, aptly termed the “leucine zipper,” is a heptad of leucine repeats that intercalate with repeats of the dimer partner, forming a coiled coiled of α-helices in parallel orientation (1–3). Electrostatic interactions between amino acids along the dimerization interface determine the specificity of dimer formation among C/EBP family members as well as with transcription factors of the NF-κB and Fos/Jun families (2). C/EBP dimerization is a prerequisite to DNA binding (4). DNA binding specificity, however, is determined by the DNA contact surface, the “basic” region of approximately 20 amino acids, upstream of the leucine zipper, specifically by three amino acids lying along the protein-DNA interface (1, 5). Domains responsible for transcriptional activation and/or repression are located in the N-terminal end of the protein.

In this review, C/EBP genes are designated C/EBPα, β, γ, -δ, -ε, -ζ as proposed by Cao et al. (6); however, Table I lists alternative nomenclature. C/EBPα was the first member cloned (7–12). Expression patterns of C/EBPα mRNA are similar in the mouse and human with measurable levels in liver, adipose, intestine, lung, adrenal gland, peripheral blood mononuclear cells, and placenta (8, 12). In liver and adipose, highest levels of C/EBPα mRNA are detected only in differentiated tissue (8, 12). Autoregulation of C/EBPα mRNA occurs by different mechanisms in the mouse and in humans. The murine C/EBPα promoter directly binds C/EBPα within 200 base pairs of the transcriptional start resulting in 3-fold activation (9). Autoregulation of the human C/EBPα promoter occurs by C/EBPα-induced binding of USF, a ubiquitously expressed transcription factor, to its upstream site within the C/EBPα promoter (13).

Two isoforms of C/EBPα are generated from its mRNA by a ribosomal scanning mechanism (14, 15). The full-length protein is 42 kDa and contains three transactivation domains (TEI–III) (16–18). TEI and TEII mediate cooperative binding of C/EBPα to TATA box-binding protein (TBP) (TATA box-binding protein) and TBP, two components of the RNA polymerase II basal transcriptional apparatus (17). TRII contains a negative regulatory subdomain (16).

A fraction of ribosomes ignore the first two AUG codons and initiate translation at the third AUG, 351 nucleotides downstream of the first AUG (14, 15). This shorter 30-kDa protein retains its dimerization and DNA-binding domains; however, it possesses an altered transactivation potential compared with the 42-kDa isoform (14, 15).

The human, mouse, and rat genes for C/EBPβ have been cloned (6, 19–23). Constitutive expression of C/EBPβ is highest in liver, intestine, lung, and adipose; however, in the mouse, it is also detectable in kidney, heart, and spleen by Northern analysis (6). Stimulation with lipopolysaccharide (LPS), IL-6, IL-1, dexamethasone, and glucagon strongly induces C/EBPβ expression, suggesting a role in the mediation of the inflammatory response (20, 24–26).

Like C/EBPα, two C/EBPβ isoforms are generated from a single mRNA by a leaky ribosomal scanning mechanism. The full-length 32-kDa protein, also termed LAP, encodes for the conserved activation domains found in other C/EBP proteins, as well as two regulatory domains, RD1 and RD2, which confer DNA binding inhibition in a cell type-specific manner (27). The truncated protein, LAP, translated from the third, in-frame AUG, possesses only the DNA-binding and leucine zipper domains (22, 28). Het eroterodimerization of the truncated isoform with the full-length C/EBPβ (LAP) attenuates transcriptional activity in substoichiometric amounts, suggesting a dominant negative mechanism of transcriptional regulation (28).

C/EBPβ was originally identified as a mediator of IL-6 signaling, binding to IL-6-responsive elements in the promoters of acute phase response genes TNF, IL-8, and G-CSF (20, 22). Signal transduction of the acute phase response by IL-1 and LPS also induces C/EBPβ transcription (20, 25). TNFα promotes nuclear localization of C/EBPβ and C/EBPδ in response to inflammatory stress (29). Cytokine stimulation further increases C/EBPβ transcriptional activity by enhanced DNA binding (22). Post-transcriptional modifications of C/EBPβ by protein kinases in the signal transduction

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1 The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CHOP, C/EBP homogenous protein; PPAR, peroxisome proliferator-activated receptor.
pathway of C/EBPβ appear to activate transcription (30, 31).

C/EBPα is a short, intronless gene, whose mRNA is ubiquitously expressed with highest levels found in non-differentiated, progenitor cells (19, 32, 33). The 16.4-kDa encoded protein possesses a leucine zipper dimerization domain and DNA-binding region; however, it lacks transcriptional transactivating elements (33). Heterodimerization with C/EBPa and C/EBPβ attenuates transcriptional activation of target genes, suggesting dominant negative regulation of C/EBP transactivation in undifferentiated, non-in-
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| Name       | Alternative name | Expression pattern                        | Knockout model | Phenotypic abnormalities                  |
|------------|-----------------|-----------------------------------------|----------------|------------------------------------------|
| C/EBPa     | C/EBP           | Liver, adipose, intestine, lung, adrenal gland, placenta, ova, etc. | Yes            | Hepatocyte proliferation, perinatal lethal |
| C/EBPβ     | NF-IL6, IL-6DBP | Liver, intestine, lung, adipose          | Yes            | Defective lipid storage, defective carbohydrate metabolism |
| C/EBPγ     | Ig/EBP, C/EBP   | Ubiquitous                              | No             | Defective lipid storage (synergistic with C/EBPβ) |
| C/EBPα     | NF-IL6, IL-6DBP | Liver, intestine, lung, adipose          | Yes            | Immunodeficient, defective Th1 response, Macrophage phagosome defect |
| C/EBPβ     | CRP1            | Myeloid and lymphoid lineages            | Yes            | None detected                            |
| C/EBPβ     | CHOP, Gadd 153  | Ubiquitous                              | Yes, in progress | Immunodeficient, granulocyte defects, myeloid proliferation |

* Peripheral blood mononuclear cells.
transcription of fatty acid synthase, GLUT4, and 422/p2 is upregulated in white adipose tissue of the C/EBPα-deficient mouse, which is inconsistent with transcriptional data from 3T3-L1 cell lines (47, 54–56). Redundant transcriptional elements operating in the animal model may regulate the fatty acid synthesis pathway, compensating for the lack of C/EBPα.

Mice deficient in both C/EBPα and C/EBPβ expire perinatally, similar to C/EBPα knockout mice (57). C/EBPα and C/EBPβ double knockout mice did not accumulate lipid droplets in brown adipose tissue and had significantly reduced epididymal fat pads in surviving adults (57). Despite these defects, C/EBPα and PPARγ expression was normal, suggesting that C/EBPα and PPARγ are not sufficient for adipocyte differentiation in the absence of C/EBPβ and C/EBPα (57).

Adipocyte differentiation into functional adipocytes results from a highly regulated cascade of C/EBP isoform expression. Dexamethasone- and methylisobutylxanthine-stimulated 3T3-L1 preadipocytes express high levels of C/EBPα and C/EBPβ. These factors diminish during the late phase of differentiation concurrent with the appearance of high levels of C/EBPβ (6, 58). Ectopic expression of C/EBPβ in 3T3-L1 cells arrests mitotic growth (59). Likewise, abrogation of C/EBPβ expression, either by antisense interactions or hydrocortisone administration, prevents terminal adipocyte differentiation (14, 60).

C/EBPs interact with known regulators of cell cycle progression; it activates transcription and induces post-transcriptional stabilization of p21(WAF1/CIP1/CDKN1) protein, an inhibitor of cyclin-dependent kinase (61, 62). Additionally, C/Myck and C/EBPβ share a reciprocal relationship, balancing proliferation versus growth arrest via C/EBPβ-transactivated expression of gadd45 (growth arrest-associated gene), a target of p53 tumor suppressor protein at G1 (63, 64).

Transient modulation of C/EBP levels in response to insulin and dexamethasone suggests a dynamic role in adipocyte metabolism (65). Induction of C/EBPβ and C/EBPα occurs within 1 h of insulin stimulation, resulting in a 20-fold increase of transcription factor levels by 4 h (65). Insulin treatment also decreased DNA binding of C/EBPα while increasing nuclear C/EBPβ and C/EBPβ binding (65). Insulin also induces rapid dephosphorylation of C/EBPα and represses C/EBPβ expression, modulating adipocyte gene transcription (e.g., GLUT4) (65, 66). Another gene target of C/EBPβ, the ob gene (67, 68), may be similarly regulated. Likewise, dexamethasone rapidly induces C/EBPβ levels, reciprocally repressing C/EBPα expression (69).

C/EBPβ regulates stress-inducible growth arrest in adipose tissue. Late in adipogenesis and during conditions of nutrient deprivation CHOP mRNA transcription is enhanced (45, 70, 71). CHOP attenuates C/EBPα and C/EBPβ activity by forming non-DNA binding heterodimers, and if expressed early in the adipogenesis program, will inhibit differentiation (45, 70). Induction of CHOP in adipocytes by cellular stress blocks G1 to S phase progression resulting in growth arrest (72).

The oncogenic variant of CHOP is found exclusively in myxoid liposarcomas (73). Chromosomal translocation of t(12;16)(q13;p11) in growth arrest (72).

Instability of G-CSF and GM-CSF receptors (74–76) and neutrophil elastase (77). TLS-CHOP fuses CHOP to an RNA-binding protein, which possesses strong liposarcomas (73). Chromosomal translocation of t(12;16)(q13;p11) in growth arrest (72).

C/EBPβ-deficient mice are highly susceptible to Candida albicans, Listeria monocytogenes, and Salmonella typhi (81, 82). Lethality from these pathogens may be in part because of macrophage defects and escape of phagocytosed bacteria from the phagosome to the cytoplasm (82). Low IL-12 levels and depressed delayed-type hypersensitivity, consistent with an impaired Th1 immune response, are seen in these mice (81). Elevated IL-6 levels, reported by one group, in C/EBPβ-deficient mice coincide with splenomegaly, peripheral lymphadenopathy, plasmacytosis, and extramedullary hematopoiesis, as seen in Castleman’s disease in humans (81).

In B cells, C/EBPβ is the predominant isoform in early cells, decreasing with cellular maturity (83). C/EBPβ becomes highly expressed in mature B cells and with LPS stimulation (83). Consistent with this observation, C/EBP sites are activators in mature B cells but not in early cells, suggesting that C/EBPβ and C/EBPγ play reciprocal roles (83).

Mice nullizygous for C/EBPβ survive only 2–5 months after birth (84). Frequently, these mice succumb to tissue effacement by immature granulocytes; however, 60% of mice typed have a systemic infection with Pseudomonas aeruginosa at time of death (84). C/EBPβ-deficient mice generate atypical hyposegregated granulocytes that are functionally defective, lacking an oxidative burst (84). Additionally, derangements in cytokine signaling are evidenced by low levels of mRNAs for interferon-γ, IL-2, IL-4, IL-12p40, and TNF-α (84). These results suggest that C/EBPβ acts temporally downstream of C/EBPα in granulopoiesis, blocking the last steps in terminal differentiation of mature segmented granulocytes.

**Other Systems—**C/EBPβ role in the function of other organ systems is only beginning to be elucidated. A significant percentage of C/EBPβ-deficient mice succumb to respiratory defects soon after birth (46). Histologic examination of C/EBPβ-deficient lungs shows hyperproliferation of type 2 pneumocytes (46). C/EBPα expression is temporally correlated with the appearance of surfactant A protein and is not present in A549 cells, a cell line that does not express surfactant proteins (85).

Normal ovarian physiology is dependent upon both C/EBPα and C/EBPβ. Rat ovarian follicles express C/EBPs in a cell-, time-, and hormonally specific manner (86). Attenuation of C/EBPβ expression results in decreased responsiveness to estrogen, gonadotropins and decreased ovulation rate (86). Additionally, attenuation of C/EBPα expression is associated with elevated expression of proto-oncogene c-myc (86). C/EBPβ mediates signal transduction of luteinizing hormone and is essential for the formation of corpora lutea (87). C/EBPβ-deficient mice fail to down-regulate expression of prostaglandin endoperoxidase synthase 2 and p450 aromatase in response to luteinizing hormone and are sterile (87).

**Conclusions**

C/EBPs act as pivotal regulators of cellular differentiation, terminal function, and response to inflammatory insult. Their extensive involvement in hepatic, adipose, and hematopoietic systems suggests the certainty of C/EBPs role in other tissues and systems. As potent mediators of gene expression, C/EBPs may be the future of some gene therapies or offer a deeper understanding of the forces driving oncogenesis. We are only beginning to understand the intricate pathways that transduce cell surface receptor signaling to gene transcription and subsequent protein activation. Future work with animal models deficient in multiple C/EBP isoforms will further elucidate these complex pathways.

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