Metabolic Response of Mice to a Postnatal Ablation of CCAAT/Enhancer-binding Protein α*

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Jianqi Yang†, Colleen M. Croniger†, Julie Lekstrom-Himes§, Pu Zhang§, Maris Fenys§, Daniel G. Tenen§, Gretchen J. Darlington, and Richard W. Hanson‡

From the †Departments of Biochemistry and Nutrition, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4935, the ‡Harvard Institutes of Medicine, Harvard University School of Medicine, Boston, Massachusetts 02115, and the §Huffington Center for Aging, Baylor College of Medicine, Houston, Texas 77030

Although CCAAT/enhancer-binding protein α (C/EBPα) is essential for initiating or sustaining several metabolic processes during the perinatal period, the consequences of total ablation of C/EBPα during postnatal development have not been investigated. We have created a conditional knock-out model in which the administration of poly(I:C) caused a virtually total deletion of c/ebpa (C/EBPα−/− mice) in the liver, spleen, white and brown adipose tissues, pancreas, lung, and kidney of the mice. C/EBPα itself was completely ablated in the liver by day 4 after the injection of poly(I:C). There was no noticeable change in phenotype during the first 15 days after the injection. The mice maintained a normal level of fasting blood glucose and responded to the diabetogenic action of streptozotocin. From day 16 onward, the mice developed hypophagia, exhibited severe weight loss, lost triglyceride in white but not brown adipose tissue, became hypoglycemic and hypoinsulinemic, depleted their hepatic glycogen, and developed fatty liver. They also exhibited lowered plasma levels of free fatty acid, triglyceride, and cholesterol, as well as marked changes in hepatic mRNA for C/EBPβ, peroxisome proliferator-activated receptor α, sterol regulatory element-binding protein 1, hydroxymethylglutaryl-coenzyme A reductase, and apolipoproteins. Although basal levels of hepatic mRNA for the cytosolic isoform of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase were reduced, transcription of the genes for these enzymes was inducible by dibutyryl cyclic AMP in C/EBPα−/− mice. The animals died about 1 month after the injection of poly(I:C). These findings demonstrate that C/EBPα is essential for the survival of animals during postnatal life and that its ablation leads to distinct biphasic change in metabolic processes.

C/EBPα is a member of the basic region-leucine zipper family of transcription factors and is abundant in white and brown adipose tissues, liver, lung, and proliferating myeloid cells; it is also found in the small intestine, adrenal gland, placenta, brain, and kidney (1–3). C/EBPα mRNA is detectable in mouse liver as early as 13–14 days of fetal life, peaks around birth, decreases in the immediate neonatal period, and rebounds in the adult (4–7). This biphasic expression is consistent with the suggested roles for C/EBPα in various cellular processes during perinatal development, e.g. the initiation of glycogen synthesis, ureagenesis, gluconeogenesis (8–10), and adipogenesis (8), and the maturation of the lung (11, 12) as well as the differentiation of neutrophils (13) and macrophages (14). C/EBPα has also been shown to repress cell proliferation (15–18) as well as to regulate granulopoiesis (19) during postnatal development.

Deletion of C/EBPα leads to hypoglycemia, as well as a delayed and reduced expression of PEPCK-C in the liver of C/EBPα−/− mice (8). Because PEPCK-C is a key enzyme in gluconeogenesis (20) and because C/EBPα binds to the PEPCK-C gene promoter at the CRE (cAMP-responsive element) and P3(1) sites (21–23), thereby stimulating transcription of the gene (21, 24), this transcription factor is thought to be involved in the control of glucose homeostasis. In murine species, the expression of the PEPCK-C gene is initiated at birth, and its level in the liver is reciprocally regulated by glucagon (acting via cAMP) and insulin. It has been suggested that C/EBPα is involved in cAMP-stimulated transcription of the gene for PEPCK-C in intact animals (25) and in hepatoma cells (24, 26, 27).

Because C/EBPα−/− mice die shortly after birth (8, 11), a number of mouse models have been developed to investigate the functional role of C/EBPα during postnatal life. These models have primarily focused on the ablation of C/EBPα in a limited number of tissues (28–30). It is, thus, not known whether the total ablation of C/EBPα during postnatal life is lethal to animals or destructive to metabolic processes. To investigate the consequence of a total deletion of c/ebpa in adult mice, we have created a mouse model in which the gene for C/EBPα was virtually totally deleted in the liver, white and brown adipose tissues, spleen, pancreas, kidney, and lung by a single injection of poly(I:C). The metabolic implications of ablating this transcription factor in animals after birth are presented.

EXPERIMENTAL PROCEDURES

Creation of Mice (Genotype = Mx1-Cre+ + C/EBPαF/F−) — Mx1-Cre mice were kindly provided by Klaus Rajewsky (31). The creation of C/EBPα−/− and C/EBPαF/F mice has been described in detail previously (8, 19). Mice (genotype = Mx1-Cre+ + C/EBPαF/F−) were created through breeding followed by selecting for the animal with the desired combination of transgenes. The genetic background of this new line is a mixture of C57BL6, SVE129, and CBA strains.

Genotyping Mice Using PCR — Transgenic mice were routinely genotyped by using a single PCR reaction to simultaneously identify three transgenes, the C/EBPα allele, the C/EBPα− allele, and the Mx1-Cre. The primers, αNeo-1 (5’-CCACCCCTTTCCAGCTC-3’) and αNeo-2 (5’-GAGGAAAGCTAGAGCCAATACC-3’), were designed to amplify a region of 710 bp in the C/EBPα− allele (see Fig. 1A). The
primers LoxP-2 (5’-CGCAGAGATGGTGGCTTCTTT-3’) and LoxP-3 (5'-ATACATTATACGAAATTCGCCGCCG-3’) were used to amplify a region of 173 bp in the C/EBPα allele (see Fig. 1A). The primers Cre-1 (5’-ATGTCTCATTTTACTGACG-3’) and Cre-2 (5’-CGCCCCGATACCGTGAACG-3’) were used to amplify a 355-bp region in the coding sequence in the Mx1-Cre transgene. The PCR reaction was carried out in a 25 μl solution containing ~250 ng of genomic DNA (1 μl) isolated from the tail of a mouse, 2.5 μl of 10× reaction buffer (PCRx Enhancer System, Invitrogen), 2 μl of 10 mM dNTP mixture (2.5 mM each), 2 μl of 25 mM MgSO4, 0.8 μl of primer mixture of αNeo (2.5 μM αNeo-1 and 5 μM αNeo-2), 4 μl of primer mixture of C/EBPα (5 μM LoxP-2 and 5 μM LoxP-3), 0.8 μl of primer mixture of Mx1-Cre transgene (5 μM Cre-1 and 5 μM Cre-2), and 1 unit of Taq DNA polymerase (Invitrogen). After denaturation at 95 °C for 2 min, the cycling reaction was performed as follows: 57 °C for 30 s, 72 °C for 50 s, 94 °C for 14 s, with a total of 35 cycles. The PCR products were resolved by 1% agarose gel electrophoresis. Three specific DNA products, corresponding to three transgenes (a 710-bp band for the Mx1-Cre transgene) were detected (see Fig. 1A) for the C/EBPα allele (516 bases of mRNA, GenBank™ NM_024125); for C/EBPα (316 bases of mRNA, GenBank™ NM_011480); for SREBP-1 (324 bases of mRNA, GenBank™ NM_011044); for glycogen synthase (1483 bases of mRNA, GenBank™ NM_012565); for C/EBPα (2168 bases of mRNA, GenBank™ NM_023114); for SREBP-1 (1831 bases of mRNA, GenBank™ NM_011480); for C/EBPα (2168 bases of mRNA, GenBank™ NM_011480); for C/EBPα (2168 bases of mRNA, GenBank™ NM_011480).

Western Blotting—Livers were isolated and fractionated into cytosolic and nuclear fractions as described by Dignam et al. (33) and used for Western blotting as previously described (34). Nuclear extracts (15 μg of protein) were separated by electrophoresis in a 12.5% SDS-PAGE gel. C/EBPα was detected by using rabbit anti-C/EBPα IgG as the primary antibody (Santa Cruz Biotechnology, SC-61; 1:1000 dilution) and goat anti-rabbit IgG-HRP as the secondary antibody (Santa Cruz Biotechnology, SC-2004; 1:3000 dilution).

Measurements of Body Weight and Food and Water Consumption—Animals were transferred to metabolic cages 2 weeks before the administration of poly(I:C) so as to become acclimatized to a new living environment. Adult animals were then given a single injection of poly(I:C) (10 mg/kg of body weight) at 3 months of age. Their initial body weight, measured before the injection, was designated as the starting body weight, and their body weight and food and water consumptions were recorded every other day. Changes in body weight were calculated as the average daily body weight minus the starting body weight of the animal. Unless indicated, all animals were given free access to water and standard chow (Prolab® 5P75 Isopro™ 3000 containing 22% protein, 5% fat, and 5% crude fiber and the remainder carbohydrate). Some control mice (genotype = Mx1-Cre-/- and C/EBPα<sup>−/−</sup>) were pair-fed, starting on day 18 after the injection of poly(I:C). They had free access to water, but their daily food consumption was rationed by using the equation y = z − w × 0.115655 + w × 16.3675 x<sup>0.276545</sup> x<sup>−1</sup>, where y is the daily food supply (g), w is the starting body weight (z), s is the food intake on day 18 after the injection of poly(I:C), and x is the number of days after the injection.

DNA Microarray Analysis—One-day-old neonates were given a single injection of poly(I:C) (10 mg/kg of body weight), and their livers were removed 28 days later. Total RNA, isolated from the liver, was analyzed by using murine MoE430A GeneChip® array (Affymetrix) at the DNA Microarray Core Facility at Case Western Reserve University, School of Medicine. Four animals, evenly divided into two groups, were analyzed simultaneously. Animals in each group were siblings of the same gender, with one C/EBPα<sup>−/−</sup> mouse and one normal-fed control animal (genotype = Mx1-Cre<sup>−/−</sup> + C/EBPα<sup>−/−</sup>). The data were grouped and analyzed with GeneSpring software program (Silicon Genetics). The probe sequences on the MoE430A array were confirmed by comparing them to the mouse genomic sequence data base at the NCBI (www.ncbi.nlm.nih.gov/genome/seq/MmBlast.html). Only probes with sequences located in the coding sequence and/or the untranslated region of the intended genes were included in our analysis. The reliability of the final data was further confirmed by verifying their reproducibility in both groups and by performing Northern analysis. The data are presented as -fold change in mRNA concentration in C/EBPα<sup>−/−</sup> mice compared with normal-fed controls (genotype = Mx1-Cre<sup>−/−</sup> + C/EBPα<sup>−/−</sup>).

Histological Analysis of Tissues—Fresh tissue was collected from mice and placed in 10% neutral-buffered formalin solution (Sigma). Hematoxylin-and-eosin staining was performed at the Histology Core Facility at Case Western Reserve University, School of Medicine, and Oil red O staining was done at the Veterinary Diagnostic Services of Marshfield Laboratories (Marshfield, WI).

Assay of Metabolites in the Blood—Mice were anesthetized by an intraperitoneal injection of Avertin (2,2,2-trichloroethanol; 0.5 ml of 20 mg/ml solution per 25 g of body weight). The concentration of glucose in the blood was determined by using Encore® Glucometer. Plasma was generated from whole blood using MICROTAENER® plasma separator tubes (BD Biosciences). The plasma concentrations of insulin, leptin, and glucagon-like peptide-1 (active) were measured with a mouse insulin enzyme-linked immunosorbert assay (ELISA) kit (Linco Research,
Inc.), a mouse leptin TiterZyme® enzyme immunoassay assay (EIA) kit (Assay Designs, Inc.), and glucagon-like peptide-1 (active) ELISA kit (Linco Research, Inc.). The measurement of triglyceride, fractionated bilirubin, albumin, β-hydroxybutyrate, total protein, blood urea nitrogen, cholesterol, and free fatty acid was performed at the Veterinary Diagnostic Services of Marshfield Laboratories (Marshfield, WI).

**Determination of Hepatic Glycogen**—Glycogen was determined according to Suzuki et al. (35) with modifications. Pieces of liver (~50 mg) were dissolved in 0.5 ml of 30% KOH. Glycogen was then precipitated with 0.5 ml of 100% ethanol and 10 µl of 4 M LiCl by centrifugation at 3000 X g for 30 min. To degrade the glycogen (in the pellet) to glucose, 0.5 ml of 4 M HCl was added to lyse the pellet, and the glucose-containing lysate was neutralized with 0.5 ml of 2 M K2CO3 solution. The concentration of glucose was determined using an YSI glucose analyzer (Giangarlo Scientific Co.).

**Hormone and Other Treatments**—Hormones were administrated to animals according to Lechner et al. (36). Briefly, streptozotocin (freshly prepared, 200 mg/kg of body weight) was administrated to mice at day 11 after the injection of poly(I:C). Mice were fasted 18 h before injection and fed 2 h later. The concentration of blood glucose was measured daily using blood collected from the tail vein. Animals with a blood glucose concentration higher than 250 mg/dl were considered diabetic. Bt2cAMP was administered to the mice at day 28 after the injection of poly(I:C). Mice were fasted 18 h before injection and fed 2 h later. The concentration of blood glucose was measured daily using blood collected from the tail vein. Animals with a blood glucose concentration higher than 250 mg/dl were considered diabetic.

**RESULTS**

**Creation and Characterization of Mice with a Genotype of Mx1-Cre+ + C/EBPαF/−**—To delete c/ebpa in adult mice, we developed a mouse model that was heterozygous for c/ebpa. One allele of c/ebpa is flanked by a loxP site (designated as C/EBPαF/−) (8). The genome of the mice also included a chimeric transgene Mx1-Cre (31), composed of the gene for Cre recombinase, linked to the Mx1 gene promoter that can be transiently activated by the administration of interferon α, interferon β, or an interferon inducer, such as poly(I:C). Use of mice with a heterozygous composition of c/ebpa (C/EBPαF/−) complicates genotyping procedures because two additional alleles (C/EBPαF/− and C/EBPα−) have to be genotyped compared with homozygous (C/EBPαF/F) mice. We, therefore, developed a PCR-based protocol that allows us to genotype the three transgenes simultaneously in a single PCR reaction. This involved the construction of three sets of primers that amplified unique regions of the C/EBPαF/−, Mx1-Cre+ + C/EBPαF/− transgenes (Fig. 1A). The sizes of PCR products generated with these primers were 710 bp for C/EBPαF/−, 355 bp for Mx1-Cre+ + C/EBPαF/−, and 173 bp for C/EBPα− (Fig. 1B). Mice with a genotype of Mx1-Cre+ + C/EBPαF/− developed normally and were indistinguishable from mice with a genotype of C/EBPαF/F (wild type), C/EBPαF/−, or C/EBPα−. An advantage of using mice with such a heterozygous genotype is that they only have 50% of c/ebpa (one allele, C/EBPαF/−) to be excised by Cre recombinase as compared with mice with two alleles of the gene (C/EBPαF/−).

**Efficiency and Specificity of the Excision of C/EBPαF in Various Tissues**—To assess the excision efficiency of C/EBPαF allele, a DNA fragment obtained after NotI digestion of the 5′-region of genomic DNA for C/EBPα (Fig. 1A) was used as a probe for Southern blotting. This probe hybridizes to the alleles of C/EBPαF+/−, C/EBPαF/F, and C/EBPαF/F, but not to C/EBPαF−, which lacks the probe sequence (Fig. 1A). Two fragments were detected by Southern blotting; they are a 9.2-kb fragment for the C/EBPαF+/− and C/EBPαF/F alleles and a 4.9-kb fragment for the C/EBPαF− allele (the excised product). After the poly(I:C) treatment, c/ebpa was totally deleted in the liver of mice that carried one copy of C/EBPαF (Fig. 2A, lanes 2–3) or two copies of C/EBPαF (Fig. 2A, lanes 1 and 4). The specificity of deletion is shown in the last four lanes of Fig. 2A. As expected, only 50% of c/ebpa was excised when one allele was C/EBPαF− and the other was C/EBPαF− (Fig. 2A, top panel, lane 8). Quantification of the Southern blots indicated 97% deletion of c/ebpa in the liver of C/EBPαF− mice and 91% deletion in C/EBPαF− mice compared with control mice (genotype = Mx1-Cre− + C/EBPαF/F) (Fig. 2B,Liv). There was also almost total deletion of c/ebpa in other tissues of C/EBPαF− mice: spleen (~90%), white adipose tissue (WAT) (~89%), pancreas (~83%), lung (~80%), kidney (~80%), and brown adipose tissue (BAT) (~80%) (Fig. 2B, filled bars). This was a marked improvement over the inefficient deletion of the gene noted in C/EBPαF− mice (Fig. 2B, hatched bars). We have, thus, created a model in which the gene for C/EBPα has been ablated in C/EBPα-abundant tissues of adult mice.

**Kinetics for the Excision of C/EBPαF in Liver**—To evaluate the rapidity of the excision of c/ebpa, the levels of DNA, mRNA, and protein for hepatic C/EBPα were measured at days 1–4 after injection of poly(I:C).

![FIGURE 1. Genotypic analyses of mice. A. Schematic alignment of four C/EBPα alleles. C/EBPαF− (knock-out allele), C/EBPαF+/− (wild type (WT) allele), C/EBPαF/F (floxed allele), and C/EBPαF− (excised product) are aligned relative to the EcoRI site (drawing is to scale). Open and gray boxes represent coding sequences of the neoR (neomycin resistance gene) and C/EBPαF− genes, respectively; arrows indicate the direction of transcription. Black triangles indicate the locations of Lox P sites. The Nott fragment, which was used as probe in Southern blotting, is represented with a hatched bar. The sizes of the hybridized fragments (resulting from digestion with EcoRI) are indicated along with each allele. Lines with diamond ends represent the locations of PCR products for C/EBPα− and C/EBPαF/F alleles. B. BamHI; E; EcoRI; EV, EcoRV; N; Nott; S; StsII; kb, kilobases. B. PCR analyses of transgenes. PCR products were resolved by electrophoresis in a 1% agarose gel. The unique product for each transgene is shown at the left side of the panel, and mouse genotype is indicated at the bottom. M, DNA size marker.](38691_1)
Approximately 95% of c/ebpa was excised from the liver within 24 h after the injection (Fig. 3A); the excision was complete by the 2nd day. Virtually no C/EBPa mRNA was detected in the liver 1 day after injection of poly(I:C) (Fig. 3B). C/EBPa protein also rapidly disappeared from the nucleus. In fact, only two isoforms, i.e. A and B1 (also known as p42) (37), were visible at days 1 and 2 after injection of poly(I:C) (Fig. 3C, lanes 3 and 4). By day 4, C/EBPa protein was completely ablated in the liver of C/EBPa<sup>−−</sup> mice (Fig. 3C, compare lanes 5 and 6 to lanes 1, 2, and 7). By comparing the intensity of B1 isoform at different time points, we estimated that the half-life of C/EBPa protein is less than 12 h in the liver of animals.

Postnatal Development and Hypophagia of C/EBPa<sup>−−</sup> Mice—To investigate the role of C/EBPa during postnatal development, we ablated C/EBPa in mice at either 1 day after birth (neonate) or at 3 months of age (adult). When poly(I:C) was administrated to neonates, the resultant C/EBPa<sup>−−</sup> mice showed no noticeable change in phenotype during the first 15 days (Phase I), after which the animals displayed marked growth retardation and died about 4 weeks after the injection of poly(I:C); their body weights were about one-third those of control mice (Fig. 4A). In an attempt to nutritionally “rescue” the animals, their regular diet was supplemented with 20% sucrose solution or liquid diet (Peptamen<sup>®</sup> complete elemental diet) during the third and fourth weeks after the injection (Phase II). This treatment mildly improved their food consumption and extended their lifespan by 1−2 weeks (Fig. 4A, dotted line). When poly(I:C) was administrated to mice at 3 months of age, the resultant C/EBPa<sup>−−</sup> mice also exhibited a biphasic change in phenotype: i.e. they were normal during the first 15 days (Phase I) but during the subsequent 15 days (Phase II) had severe weight loss (Fig. 4B). hypo-
Postnatal Ablation of C/EBPα

Ablation of C/EBPα Alters the Deposition of Triglyceride in Tissues—C/EBPα knockout mice lost triglyceride in their white but not brown adipose tissue (Fig. 5, A and B) at the end of Phase II irrespective of whether C/EBPα was ablated at 1 day or 3 months of age. Correspondingly, the size of the white adipose tissue was substantially smaller than in normal- and pair-fed controls (Fig. 5C), but the brown adipose tissue was either larger than in normal-fed controls (injected with poly(I:C) at 1 day of age) or similar to that in normal- and pair-fed controls (injected with poly(I:C) at 3 months of age) (Fig. 5C). Additionally, C/EBPα−/− mice, but not normal- or pair-fed controls, developed a marked fatty liver such that lipid infiltration was visible as white spots on the surface of the liver (Fig. 5D). Thus, C/EBPα is essential for maintaining the differentiated status of white but not brown adipose tissue in adult mice, and its ablation led to an abnormal deposition of fat in liver.

Impaired Energy Homeostasis in the C/EBPα−/− Mice—Based on the observed hypophagia, C/EBPα−/− mice are likely in an "energy crisis" during Phase II. Various energy-yielding metabolites in the blood were measured 28 days after the injection of poly(I:C). The concentrations of glucose, free fatty acids, and triglyceride were markedly lower (<50%) in C/EBPα−/− mice as compared with normal-fed controls irrespective of whether the ablation of C/EBPα was performed on neonates or 3-month-old adults (TABLE ONE). The concentration of β-hydroxybutyrate in the plasma of C/EBPα−/− mice was normal when ablation of C/EBPα was carried out 1 day after birth but was higher than in controls if the deletion was performed at 3 months of age (TABLE ONE). Ablation of C/EBPα also caused a decrease in cholesterol and an increase in total protein levels in the blood as compared with normal- or pair-fed controls. The decrease in levels of serum albumin and blood urea nitrogen appears to be dependent on the time of ablation of C/EBPα. The hyperbilirubinemia, noted by Lee et al. (28) in their mice was not observed in our study (TABLE ONE) even though the level of mRNA for UDP-glucuronosyltransferase-1, the enzyme required for the conjugation of glucuronic acid to bilirubin, was reduced in the liver of C/EBPα−/− mice to 40% that of the control values (TABLE TWO).

Next, we assessed the levels of hormones that regulate food intake. Surprisingly, the concentrations of leptin and glucagon-like peptide-1 were not elevated in the C/EBPα−/− mice; the level of insulin was undetectable (injection of poly(I:C) at 1 day of age) or strikingly low (injection

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was used to calculate the food supply for pair-fed controls (genotype = Mx1-Cre−/C/EBPα−/−), where y is the daily food supply (g), x is the starting body weight, z is the food intake on day 18 after the injection of poly(I:C), and c is the number of days after the injection.

Taking together, these results indicate that ablation of C/EBPα leads to a biphasic response in metabolic phenotype and eventual death of the animals regardless of whether the gene was deleted early in life (neonates) or at 3 months of age.

To examine whether hypophagia was responsible for the severe weight loss noted in C/EBPα−/− mice, control mice (genotype = Mx1-Cre−/C/EBPα+/+), were pair-fed starting at day 18 after the injection of poly(I:C). The animals had free access to water, but their daily food consumption was rationed. An algorithm generated from the daily food consumption by the C/EBPα−/− mice (Fig. 4, dotted line) was used to calculate the quantity of food supplied daily to the pair-fed controls. The pair-fed controls exhibited a similar rate of weight loss as the C/EBPα−/− mice during the pair-feeding period (Fig. 4B, dotted line), suggesting that hypophagia caused the weight loss observed in the C/EBPα−/− mice. Although the pair-fed control mice lost weight, they did not exhibit the same severe metabolic abnormalities as did the C/EBPα−/− mice.

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Postnatal Ablation of C/EBPα

The genotypes of control animals are Mx1-Cre<sup>−/−</sup> + C/EBPα<sup>F/F</sup>. A, lipodystrophy in the white adipose tissue (WAT) of C/EBPα<sup>Δ−/−</sup> mice. The abdominal area of mice, which received an injection of poly(I:C) 1 day after birth, is shown on the left side; epididymal fat from mice injected with poly(I:C) at 3 months of age is aligned on the right side. Lipodystrophy is indicated by the disappearance of triglyceride in the subcutaneous, peri-ovarian, and epididymal white adipose tissues in C/EBPα<sup>Δ−/−</sup> mice. B, relatively normal brown adipose tissue of C/EBPα<sup>Δ−/−</sup> mice. C, metabolic changes in C/EBPα<sup>Δ−/−</sup> mice. C/EBPα<sup>Δ−/−</sup> mice also had a marked decrease in the levels of hepatic mRNA for proteins that are involved in fatty acid oxidation and lipid transport (i.e. apolipoproteins) (TABLE TWO). Such changes suggest that a decreased rate of both fatty acid oxidation and lipoprotein export are involved in the lipid infiltration observed in the livers of C/EBPα<sup>Δ−/−</sup> mice. A decrease in the level of mRNA for hydroxymethylglutaryl-coenzyme A reductase (TABLE TWO) is also consistent with the reduced concentration of cholesterol noted in the blood of C/EBPα<sup>Δ−/−</sup> mice (TABLE ONE). In addition to the changes in mRNAs for various enzymes, there were marked alterations in mRNA levels of several transcription factors: C/EBPα (7-fold decrease) (TABLE TWO, Fig. 7C). The consequence of these large changes in the mRNA levels of these transcription factors remains to be further investigated.

Effect of the Ablation of C/EBPα on Glucose Homeostasis—To investigate how the ablation of C/EBPα in adult mice affects glucose homeostasis, the concentration of glucose in blood was measured. C/EBPα<sup>Δ−/−</sup> mice maintained normal blood glucose levels in fed or fast-
### TABLE ONE
Profile of metabolites in the plasma of C/EBPαKO mice and littermate controls

| Parameter measured | Injection of poly(I:C) at 1 day old | Injection of poly(I:C) at 3 months old |
|--------------------|-------------------------------------|----------------------------------------|
|                    | Normal-fed (n) | Control (n) | p Value | Normal-fed (n) | Control (n) | p Value | Normal-fed (n) | Control (n) | p Value |
| Glucose (mg/dl)    | C/EBPαKO       | Control      | 48.7 ± 6.3 (3) | 192.0 ± 13 (6) | <0.01*       | 63.3 ± 7.5 (3) | 189.7 ± 10 (8) | <0.01*       | 153.4 ± 7.2 (7) | <0.01*       |
| Free fatty acid (mEq/l) | C/EBPαKO | Control      | 0.18 ± 0.04 (3) | 0.89 ± 0.22 (5) | <0.05*       | 0.22 ± 0.05 (5) | 0.50 ± 0.09 (13) | <0.08       | 0.62 ± 0.10 (7) | =0.01*       |
| Triglycerides (mg/dl) | C/EBPαKO | Control      | 29.3 ± 10.9 (3) | 103 ± 16.4 (7) | <0.02*       | 53.2 ± 12.6 (5) | 126.8 ± 12 (13) | <0.01*       | 85.1 ± 28.5 (7) | <0.39       |
| β-hydroxybutyrate (mg/dl) | C/EBPαKO | Control      | 3.73 ± 1.04 (3) | 3.04 ± 1.05 (7) | <0.71       | 6.92 ± 4.67 (5) | 1.67 ± 0.21 (13) | <0.08       | 3.47 ± 0.54 (7) | =0.40       |
| Cholesterol (mg/dl) | C/EBPαKO       | Control      | 51.5 ± 6.5 (3) | 111 ± 13.0 (7) | <0.05*       | 65.0 ± 9.2 (5) | 109.5 ± 6.7 (13) | <0.01*       | 106.0 ± 10 (7) | =0.02*       |
| Total protein (g/dl) | C/EBPαKO | Control      | 6.40 ± 0.00 (2) | 6.43 ± 0.19 (6) | <0.01*       | 6.12 ± 0.55 (5) | 4.61 ± 0.10 (13) | <0.01*       | 4.79 ± 0.19 (7) | =0.03*       |
| Albumin (g/dl)     | C/EBPαKO       | Control      | 1.83 ± 0.22 (3) | 3.18 ± 0.15 (7) | <0.01*       | 2.88 ± 0.23 (5) | 2.92 ± 0.07 (13) | =0.81       | 3.36 ± 0.10 (7) | =0.06       |
| RUN (mg/dl)         | C/EBPαKO       | Control      | 20.7 ± 3.0 (3) | 24.9 ± 1.6 (7) | <0.21       | 26.2 ± 3.6 (5) | 36.4 ± 2.14 (13) | <0.04*       | 29.1 ± 3.0 (7) | <0.54       |
| Total bilirubin (mg/dl) | C/EBPαKO | Control      | 0.47 ± 0.12 (3) | 0.31 ± 0.05 (7) | <0.20       | 0.24 ± 0.05 (5) | 0.22 ± 0.05 (13) | <0.80       | 0.24 ± 0.04 (7) | =0.96       |
| Albumin (mg/dl)     | C/EBPαKO       | Control      | 0.40 ± 0.13 (3) | 0.26 ± 0.04 (7) | <0.21       | 0.17 ± 0.06 (5) | 0.13 ± 0.05 (13) | <0.71       | 0.14 ± 0.04 (7) | =0.71       |
| Insulin (ng/ml)     | C/EBPαKO       | Control      | BD (3)         | 0.67 ± 0.17 (7) | 0.36 ± 0.04 (5) | 0.85 ± 0.16 (8) | <0.04*       | ND         | ND         |
| Leptin (ng/ml)      | C/EBPαKO       | Control      | 0.66 ± 0.03 (5) | 2.47 ± 1.02 (3) | <0.05*       | 0.92 ± 0.14 (4) | 1.53 ± 0.52 (3) | <0.25       | ND         | ND         |
| Glucagon-like peptide-1 (pm) | C/EBPαKO | Control      | 1.93 ± 0.76 (3) | 5.28 ± 0.66 (3) | <0.03*       | ND         | ND         | ND         | ND         |

* Statistical significance.
* BD, Below detection.
* ND, not determined.

### TABLE TWO
Altered expression of selected genes in the livers of C/EBPαKO mice

| Classification       | Gene                  | -Fold change | GenBank™ accession no. | Verification using Northern blotting |
|----------------------|-----------------------|--------------|------------------------|--------------------------------------|
| Transcription factors| C/EBPα                | 3.7          | 9.8                    | A1642132                             | This study |
|                      | Jun-B oncogene        | 2.5          | 3.7                    | NM_008416.1                          |           |
|                      | c-Jun                 | 1.6          | 2.7                    | NM_010591.1                          | Ref. 11   |
|                      | C/EBPβ                | 1.2          | 1.4                    | NM_009883.1                          | This study |
|                      | PPARα                 | -4.3         | -6.7                   | BC016892.1                           | This study |
|                      | SREBP 1               | -2.9         | -11.1                  | A1326423                             | This study |
| Carbohydrate         | PEPCK-C               | -1.8         | -1.5                   | NM_011044.1                          | Refs. 8 and 28; this study |
| metabolism           | Glucokinase           | -1.3         | -6.7                   | L38990.1                             | Ref 29; this study |
|                      | Glycogen synthase     | -2.3         | -1.8                   | BC02132.2                            | Refs. 8 and 28; this study |
| Cholesterol biosynthesis | HMG-CoA reductase    | -3.0         | -4.0                   | BC019782.1                           |           |
| Urea cycle           | Ornithine transcarbamylase | -2.6      | -15.6                  | A1786408                             | Refs. 10 and 29 |
| Fatty acid oxidation | Fatty acid CoA ligase, long chain 2 | -6.5 | -4.4                   | NM_007981.1                          |           |
|                      | Enoyl CoA hydratase, short chain 1 (mito) | -2.1 | -3.2                   | B1114146                             |           |
|                      | Hydroxyacyl-CoA dehydrogenase, short chain | -2.6 | -6.3                   | NM_008212.1                          |           |
|                      | Hydroxyacyl-CoA dehydrogenase type II | -1.6 | -2.9                   | NM_016763.1                          |           |
|                      | Ketoacyl-CoA thiolase B | -12.5       | -7.7                   | BC019882.1                           |           |
| Lipid transport      | Apolipoprotein A-4    | -4.0         | -6.3                   | AV027367                             | Ref. 29   |
|                      | Apolipoprotein C-3    | -2.6         | -4.3                   | BC021776.1                           | Ref. 29; this study |
|                      | Apolipoprotein C-4    | -2.0         | -5.0                   | BC024657.1                           | Ref. 29   |
| Bilirubin             | UDP-glucuronosyltransferase 1 | -1.3 | -3.8                   | D87867.1                             | Ref. 28   |

* Mean values of two experiments (Exp. 1 and 2).
* The average -fold changes, noted in the DNA array analysis, were confirmed with the data from Northern blotting that were performed in this and other studies.

(18 h) conditions during Phase I (Fig. 8A). However, during the third week after injection of poly(I:C), their ability to maintain a normal concentration of blood glucose was attenuated, and it was severely impaired in the fourth week. Most of the C/EBPαKO mice could not tolerate an 18-h fast in the third week. C/EBPαKO mice that had free access to food lived up to the fourth week, but their fed blood glucose concentration was 30–40% that noted in the normal- or pair-fed controls (Fig. 8A, TABLE TWO). To test whether C/EBPα is required for the normal response to a diabetogenic stimulus, we injected C/EBPαKO mice with streptozotocin at 11 days after the injection of poly(I:C) to induce diabetes. The C/EBPαKO mice became diabetic in a manner similar to the control animals (genotype = Mx1-Cre + C/EBPα KO) (Fig. 8B). Next, the levels of mRNA for key regulatory enzymes that influence glucose homeostasis were determined at selected intervals after the injection of
C/EBPα Is Optional for Bt2cAMP-stimulated Transcription of PEPCK-C in the Liver of Adult Mice—The mRNA for PEPCK, one of the key enzymes in hepatic and renal gluconeogenesis, is undetectable in the liver of C/EBPα−/− mice at birth (8, 11), and in such animals its mRNA cannot be induced by the administration of Bt2cAMP (25). It has been suggested that C/EBPα is required for the transcriptional response of the gene for PEPCK-C to the cyclic nucleotide (25–27). To further investigate this possibility Bt2cAMP was administrated to C/EBPα−/− mice. The result was an increase in the levels of mRNAs for PEPCK-C and glucose-6-phosphatase (Fig. 9) to the same extent as noted in the livers of normal-fed controls, although the basal levels of mRNAs for both enzymes were much lower in C/EBPα−/− mice (Fig. 9). This finding suggests that in livers of adult mice C/EBPα is critical for the basal but not the cAMP-stimulated induction of gluconeogenic enzymes, such as PEPCK-C and glucose-6-phosphatase.

DISCUSSION

Advantages of Our Animal Model for Metabolic Studies—A number of major metabolic defects noted in C/EBPα-deficient mice, such as hypoglycemia and hyperammonemia, have established C/EBPα as critical for the perinatal development of metabolic processes (8–10). In the absence of C/EBPα, the animals die within 30 min after birth. It is not clear, however, how important this transcription factor is after the perinatal period. One line of evidence suggests that C/EBPβ can partially replace C/EBPα in the liver (7) and hematopoietic cells (38) but not in white adipose tissue (7), if the expression of C/EBPβ is controlled by an endogenous C/EBPα gene promoter. The fact that one isoform of C/EBP can substitute for another complicates the effort to examine the role of function of C/EBPα in animals. Another problem is that most animal models in which the gene for C/EBPα has been ablated have mainly focused on liver (28, 29) or adipose tissue (30). Although such models provide valuable insights into the role of C/EBPα in those tissues, they provide no information on the effect of a total loss of C/EBPα activity in the whole animal.

In this study we have created an animal model that has several advantages for studying the role of C/EBPα in the postnatal development of metabolic processes. First, the ablation of C/EBPα occurs in multiple tissues of adult mice, particularly in C/EBPα-rich tissues such as liver, white and brown adipose tissue, lung (Fig. 2), and bone marrow (19). Accordingly, the loss of function of C/EBPα was studied in the whole animal rather than in individual tissues. Second, the ablation of c/ebpα is
The Two-phase Response of C/EBPα—Ablation of C/EBPα has been proposed to be a “central regulator of energy metabolism” (41), presumably because of its participation in the differentiation of adipose tissue and its regulation of glucose homeostasis and other critical metabolic processes. The results of our study are consistent with this proposition. Ablation of C/EBPα in adult mice clearly creates an energy crisis, which results from the extremely low level of energy reserves available to the animals in Phase II. For example, the C/EBPα−/− mice have no hepatic glycogen, lose triglyceride in white adipose tissue, and develop hypophagia and fatty liver. An extreme manifestation of such crisis is the co-existence of hypoinsulinemia and hypoglycemia (TABLE ONE). Normally, a low level of insulin is accompanied by hyperglycemia, since a lack of insulin impairs glucose uptake.

Because the ablation of C/EBPα is rapid and extensive in most of the tissues studied (its half-life is less than 12 h in the liver) (Fig. 3), our current working model to explain the two-phase response of mice is that ablation of C/EBPα causes some major molecular alterations, which fully manifest themselves within a 2-week period after ablation of C/EBPα (length of Phase I); these alteration consequently lead to metabolic derangements seen in Phase II and the ultimate death of the animals. It is unlikely that these molecular alterations occur only in the liver, since excision of hepatic c/ebp either at birth (29) or in the adult (28) does not result in the death of animals. Hypophagia is also unlikely to be a major factor, since pair-feeding the animals caused weight loss but not the other abnormalities noted in our study. Because C/EBPα normally controls the transcription of many genes involved in both metabolism and cellular differentiation (39, 40), its absence probably initiates a pleiotropic response in a variety of tissues, not just the liver. The accumulated effect of alterations in the transcription of critical genes then leads to the general decline in health of the animal, as noted in Phase II. Interestingly, Zhang et al. (19) have deleted c/ebp in mice 2 days after birth and noted the obstruction of granulocyte development and a 30-fold accumulation of blasts in bone marrow. They suggested that their C/EBPα-deficient mice (C/EBPα−/− mice) died from sepsis as a result of granulocytopenia. This raises a possibility that C/EBPα−/− mice also die from sepsis, because the degree of ablation of C/EBPα in the bone marrow of C/EBPα−/− mice should be close to, if not greater than that in C/EBPα−/− mice. However, we have noted that the onset of hypophagia in the C/EBPα−/− mice occurs at almost exactly the third week after ablation of c/ebp. This might explain why this model of sepsis develops in a virtually synchronous manner in the mice. We also determined the body temperature of C/EBPα−/− mice both at room temperature and at 4 °C and found no difference from littermate controls. Also, it is possible that the large accumulation of blasts produce cytokines that selectively alter metabolic processes and, subsequently, a decrease in appetite. The above possibilities need to be investigated.

C/EBPα and the Postnatal Development of Metabolic Processes—C/EBPα is required in multiple tissues to maintain normal metabolic and developmental processes. We consistently noted a biphasic response of mice to the loss of C/EBPα, no matter whether deletion of the gene occurred at 1 day or 3 months after birth. C/EBPα is known to control the transcription of a number of genes and is thus likely to have a direct effect on global gene transcription (39, 40). We noted few overt metabolic alterations or changes in mRNA levels in the C/EBPα−/− mice during Phase I. In Phase II, however, there was severe deterioration of health/metabolism, which was apparently associated with marked changes in mRNA levels for the enzymes that are directly involved in hepatic metabolism, such as PEPCK-C, glucose-6-phosphatase, glycogen synthase, and glucokinase. We also noted alterations in the concentrations of mRNAs for transcription factors such as C/EBPα, SREBP-1, and PPARα, which regulate expression of genes coding for metabolically important proteins. The promoters of these genes have multiple C/EBPα-binding sites, which suggests that ablation of C/EBPα is responsible (either directly or indirectly) for the altered concentration of mRNA of these genes. It is not clear why the loss of C/EBPα causes an increase in the levels of C/EBPα and c-Jun. The simplest explanation is that C/EBPα inhibits transcription of the genes for these proteins, although this remains to be tested.

Because the ablation of C/EBPα was rapid and extensive in most of the tissues studied (its half-life is less than 12 h in the liver) (Fig. 3), our current working model to explain the two-phase response of mice is that ablation of C/EBPα causes some major molecular alterations, which fully manifest themselves within a 2-week period after ablation of C/EBPα (length of Phase I); these alteration consequently lead to metabolic derangements seen in Phase II and the ultimate death of the animals. It is unlikely that these molecular alterations occur only in the liver, since excision of hepatic c/ebp either at birth (29) or in the adult (28) does not result in the death of animals. Hypophagia is also unlikely to be a major factor, since pair-feeding the animals caused weight loss but not the other abnormalities noted in our study. Because C/EBPα normally controls the transcription of many genes involved in both metabolism and cellular differentiation (39, 40), its absence probably initiates a pleiotropic response in a variety of tissues, not just the liver. The accumulated effect of alterations in the transcription of critical genes then leads to the general decline in health of the animal, as noted in Phase II. Interestingly, Zhang et al. (19) have deleted c/ebp in mice 2 days after birth and noted the obstruction of granulocyte development and a 30-fold accumulation of blasts in bone marrow. They suggested that their C/EBPα-deficient mice (C/EBPα−/− mice) died from sepsis as a result of granulocytopenia. This raises a possibility that C/EBPα−/− mice also die from sepsis, because the degree of ablation of C/EBPα in the bone marrow of C/EBPα−/− mice should be close to, if not greater than that in C/EBPα−/− mice. However, we have noted that the onset of hypophagia in the C/EBPα−/− mice occurs at almost exactly the third week after ablation of c/ebp. This would explain why this model of sepsis develops in a virtually synchronous manner in the mice. We also determined the body temperature of C/EBPα−/− mice both at room temperature and at 4 °C and found no difference from littermate controls. Also, it is possible that the large accumulation of blasts produce cytokines that selectively alter metabolic processes and, subsequently, a decrease in appetite. The above possibilities need to be investigated.

C/EBPα and the Postnatal Development of Metabolic Processes—C/EBPα has been proposed to be a "central regulator of energy metabolism" (41), presumably because of its participation in the differentiation of adipose tissue and its regulation of glucose homeostasis and other critical metabolic processes. The results of our study are consistent with this proposition. Ablation of C/EBPα in adult mice clearly creates an energy crisis, which results from the extremely low level of energy reserves available to the animals in Phase II. For example, the C/EBPα−/− mice have no hepatic glycogen, lose triglyceride in white adipose tissue, and develop hypophagia and fatty liver. An extreme manifestation of such crisis is the co-existence of hypoinsulinemia and hypoglycemia (TABLE ONE). Normally, a low level of insulin is accompanied by hyperglycemia, since a lack of insulin impairs glucose uptake.

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**Figure 7:** Hepatic mRNA levels for selected genes in C/EBPα−/− mice. Livers were obtained from mice on day 28 after a single injection of poly(I:C) (10 mg/kg of body weight). Total RNA was isolated, and Northern blotting was carried out with cDNA probes specific for mRNA of selected enzymes (A) or transcription factors (B). The values for normal-fed control animals were arbitrarily set as 1.0. The results are expressed as means ± S.E. for four to six animals. Glc-6-Pase, glucose-6-phosphatase; GS, glycogen synthase.

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**The Two-phase Response of C/EBPα—Ablation of C/EBPα has been proposed to be a “central regulator of energy metabolism” (41), presumably because of its participation in the differentiation of adipose tissue and its regulation of glucose homeostasis and other critical metabolic processes. The results of our study are consistent with this proposition. Ablation of C/EBPα in adult mice clearly creates an energy crisis, which results from the extremely low level of energy reserves available to the animals in Phase II. For example, the C/EBPα−/− mice have no hepatic glycogen, lose triglyceride in white adipose tissue, and develop hypophagia and fatty liver. An extreme manifestation of such crisis is the co-existence of hypoinsulinemia and hypoglycemia (TABLE ONE). Normally, a low level of insulin is accompanied by hyperglycemia, since a lack of insulin impairs glucose uptake.
Postnatal Ablation of C/EBPα

**FIGURE 8.** Glucose homeostasis in C/EBPα−/− mice. A, glucose concentration in the blood. Glucose levels in the blood were measured in mice at various times after they received a single injection of poly(I:C) (10 mg/kg body weight) at the age of 3 months. The values are expressed as means ± S.E. for the number of animals indicated on the top of each column. Gray bars, control animals (genotype = Mx1-Cre+/C/EBPα+/−); black bars, C/EBPα−/− mice; hatched bars, pair-fed controls (genotype = Mx1-Cre−/C/EBPα−/−). B, streptozotocin-induced diabetes in C/EBPα−/− mice. Streptozotocin (STZ) was administrated to mice at day 11 after injection of poly(I:C). The glucose level was then measured daily using blood that was collected from the tail vein. The values are expressed as means ± S.E. for three animals in each group. C, biphonic expression profile of selected genes in the liver of C/EBPα−/− mice. Mice were killed at day 2, 8, and 22 after the injection of poly(I:C). For the 2- and 8-day time points, the mice were fasted 18 h before killing; mice analyzed at day 22 were fed. Total RNA was isolated from liver, and the expression of selected genes was determined by Northern blotting. The mouse genotype (before the injection) is indicated at the bottom of the figure. Glc-6-Pase, glucose-6-phosphatase; GS, glycogen synthase.

in peripheral tissues and leads to an increased hepatic gluconeogenesis. However, C/EBPα−/− mice consume abnormally low amounts of dietary glucose (resulting in hypophagia) and probably carry out decreased gluconeogenesis due to reduced levels of mRNA for gluconeogenic enzymes. Aberrations in both carbohydrate and lipid metabolism in the C/EBPα−/− mice likely underlie the energy crisis apparent in these animals. The exact trigger for these changes remains to be determined.

Although C/EBPα is known to be essential for adipogenesis in white and brown adipose tissues (7, 8, 30, 42), it is not clear whether it is required to maintain the differentiated status of these tissues. Because C/EBPα−/− mice have severe lipodystrophy in their white adipose tissue regardless of the time of ablation, C/EBPα must be essential not only for postnatal development but also for the maintenance of the differentiated state of the white adipose tissue. Hypophagia appears to accelerate these processes, but it is not the cause, since pair-fed controls had relatively normal white adipose tissue. In agreement with earlier studies (7, 30), the postnatal development of brown adipose tissue is not dependent on C/EBPα. Interestingly, fat deposition in the liver of C/EBPα−/− mice appears to be caused by an impaired hepatic function, likely resulting from decreased rates of hepatic fatty acid oxidation and apolipoprotein synthesis. This conclusion is consistent with the lowered levels of triglyceride and free fatty acids in the blood of C/EBPα−/− mice.

During the perinatal period C/EBPα is thought to control glucose homeostasis, presumably by regulating the transcription of genes coding for key gluconeogenic enzymes, such as PEPCK-C and glucose-6-phosphatase. Hepatic PEPCK-C is regulated at both basal (8) and stimulated (25) levels by C/EBPα during the perinatal period. Beyond the perinatal period, the importance of C/EBPα in the regulation of glucose homeostasis is not clear. In one study, Lee et al. (28) deleted the gene for C/EBPα in the liver of adult mice using an adenoviral vector expressing Cre recombinase and noted a decrease in PEPCK-C mRNA. In another study Inoue et al. (29) reported no change in hepatic PEPCK-C mRNA when c/ebpα was deleted in the liver of neonatal mice by Cre recombinase produced from the transgene of albumin-Cre. In the present study we have shown that the expression of hepatic PEPCK-C mRNA is biphonic; i.e. normal in Phase I but reduced dramatically during Phase II.
In addition, the expression of PEPCK-C can still be stimulated to the same extent as noted in controls by the administration of Bt2cAMP even though the basal level of expression for PEPCK-C is strikingly lower than noted in the livers of control mice (Fig. 9). Thus, C/EBPα is important for basal transcription of the gene for PEPCK-C but not for its induction by the cyclic nucleotide in adult liver. Perhaps another member of the basic region-leucine zipper family of transcription factors (e.g. C/EBPβ, C/EBPδ, or CREB) partially assumes the function of C/EBPα in the liver of the C/EBPαΔ-lo mice.

Finally, the phenotypic differences between C/EBPαΔ-lo mice and the mice generated by Chen et al. (7) raise another interesting point, i.e. the time of C/EBP expression is important to the development of metabolic processes. When C/EBPβ is expressed under the control of the endogenous promoter of C/EBPα, it can functionally replace C/EBPα in liver (7) or hematopoietic cells (38). However, unchanged mRNA levels of C/EBPβ in postnatal development does not fully compensate for the loss of C/EBPα in C/EBPαΔ-lo mice. Thus, when and where the gene for C/EBPα is expressed are critical for the normal development of the mouse. For example, C/EBPαΔ-lo mice die within 30 min after birth if c/ebpα is missing during fetal development (8, 11). In contrast, if the gene is deleted 1 day after birth, the animals live for about 30 days. Because the promoter of the C/EBPα gene is deleted 1 day after birth, the animals live for about 30 days. However, unchanged mRNA levels of C/EBPβ in postnatal development does not fully compensate for the loss of C/EBPα in C/EBPαΔ-lo mice. Thus, when and where the gene for C/EBPα is expressed are critical for the normal development of the mouse. For example, C/EBPαΔ-lo mice die within 30 min after birth if c/ebpα is missing during fetal development (8, 11). In contrast, if the gene is deleted 1 day after birth, the animals live for about 30 days. Because the promoter of the C/EBPα gene is functional as early as fetal day 13, C/EBPα is likely required for the initial transcription of genes that allow the animal to survive the perinatal period, especially as patternning of metabolic processes is probably completed between day 13 of fetal life and birth (8–11). After this time, ablating C/EBPα, whether it is at 1 day after birth or 3 months later, has the same consequence (i.e. death within 30 days). This underlines the key role of developmentally appropriate expression of genes that code for transcription factors, since they have a broad array of effects.

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REFERENCES

1. Birkenmeier, E. H., Gwynn, B., Howard, S., Jerry, J., Gordon, J. I., Landschulz, W. H., and Mc Knight, S. L. (1998) Genes Dev. 3, 1146–1156
2. Xanthopoulos, K. G., Mirkovitch, J., Decker, T., Kuo, C. F., and Darnell, J. E., Jr. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4117–4121
3. Scott, L. M., Civan, C. I., Borth, P., and Friedman, A. D. (1992) Blood 80, 1725–1735
4. Kuo, C. F., Xanthopoulos, K. G., and Darnell, J. E., Jr. (1990) Development 109, 473–481
5. Nagy, P., Bisgaard, H. C., and Thorgerirsson, S. S. (1994) J. Cell Biol. 126, 223–233
6. Diehl, A. M., Michaelson, P., and Yang, S. Q. (1994) Gastroenterology 106, 1625–1637
7. Chen, S. S., Chen, J. F., Johnson, P. F., Muppala, V., and Lee, Y. H. (2000) Mol. Cell. Biol. 20, 7292–7299
8. Wang, N. D., Finegold, M. J., Heydari, A., Bilyeu, T. A., Finegold, M. J., Mohamedali, K., Richardson, A., and Darlington, G. J. (1998) Nucleic Acids Res. 26, 3293–3299
9. Wang, H., Jakova, P., Wilde, M., Welm, A., Goode, T., Roesler, W. J., and Timchenko, N. A. (2001) Mol. Cell 8, 817–828
10. Zhang, P., Iwasaki-Arai, J., Iwasaki, H., Fenyus, M. L., Dayaram, T., Owens, B. M., Shigematsu, H., Lentzini, E., Huettner, C. S., Lekstrom-Himes, J. A., Akashi, K., and Tenen, D. G. (2004) Immunity 21, 853–863
11. Chakravarty, K., Cassuto, H., Reszel, L., and Hanson, R. W. (2005) Crit. Rev. Biochem. Mol. Biol. 40, 129–154
12. Park, E. A., Roesler, W. J., Liu, J., Klemm, D. J., Gurney, A. L., Thabet, J. D., Shuman, J., Friedman, A., and Hanson, R. W. (1990) Mol. Cell. Biol. 10, 6264–6272
13. Park, E. A., Gurney, A. L., Nizeliez, S. E., Hikami, P., Cao, Z., Moormann, A., and Hanson, R. W. (1993) J. Biol. Chem. 268, 613–619
14. Liu, J., and Hanson, R. W. (1991) Mol. Cell. Biochem. 104, 89–100
15. Crosson, S. M., and Roesler, W. J. (2000) J. Biol. Chem. 275, 5804–5809
16. Croniger, C., Trou, M., Lysek-Stupp, K., Cohen, H., Liu, Y., Darlington, G. J., Poli, V., Hanson, R. W., and Reszel, L. (1997) J. Biol. Chem. 272, 26306–26312
17. Wilson, H. L., McFe, P. J., and Roerel, W. J. (2001) Mol. Cell. Endocrinol. 181, 27–34
18. Wilson, H. L., and Roerel, W. J. (2002) Mol. Cell. Endocrinol. 188, 15–20
19. Lee, Y. H., Sauer, B., Johnson, P. F., and Gonzalez, F. J. (1997) Mol. Cell. Biol. 17, 6014–6022
20. Inoue, Y., Inoue, J., Lambert, G., Yim, S. H., and Gonzalez, F. J. (2004) Biol. Cell. 107, 474–478
21. Linhart, H. G., Ishinoura-Oka, K., DeMayo, F., Kibe, T., Repka, D., Poindexter, B., Bick, R. J., and Darlington, G. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12532–12537
22. Kuhn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995) Science 269, 1427–1429
23. Croniger, C. M., Millward, C., Yang, J., Kawai, Y., Arinze, I. J., Liu, S., Harada-Shiba, M., Chakravarty, K., Friedman, J. E., Poli, V., and Hanson, R. W. (2001) J. Biol. Chem. 276, 629–638
24. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
25. Yang, J., Kawai, Y., Hanson, R. W., and Arinze, A. J. (2001) J. Biol. Chem. 276, 25742–25752
26. Suzuki, Y., Lanner, C., Kim, J. H., Vilardo, P. G., Zhang, H., Yang, J., Cooper, L. D., Steele, M., Kennedy, A., Bock, C. B., Scrimgeour, A., Lawrence, J. C., Jr., and DePaoli-Roach, A. A. (2001) Mol. Cell 21, 2683–2694
27. Lechner, P. S., Croniger, C. M., Hikami, P., Millward, C., Fechter, A., Yun, J. S., and Hanson, R. W. (2001) J. Biol. Chem. 276, 22675–22679
28. Califhoven, C. F., Muller, C., and Leutz, A. (2000) Genes Dev. 14, 1920–1932
29. Jones, L. C., Lin, M. L., Chen, S. S., Krug, U., Hofmann, W. K., Lee, S., Yim, S. H., and Kronfeller, H. P. (2002) Blood 99, 2032–2036
30. Takiguchi, M. (1998) Int. J. Exp. Pathol. 79, 369–391
31. Ramji, D. P., and Foka, P. (2002) Biochem. J. 365, 561–575
32. Mc Knight, S. L., Lane, M. D., and Gluecksohn-Waelsch, S. (1989) Genes Dev. 3, 2021–2024
33. Moitra, J., Mason, M. M., Olive, M., Krylov, D., Gasvirolo, O., Marcus-Samuels, B., Feigenbaum, L., Lee, E., Aoyama, T., Eckhaus, M., Reitman, M. L., and Vinson, C. (1998) Genes Dev. 12, 3168–3181