COMMENTARY

Is CCAAT/enhancer-binding protein a central regulator of energy metabolism?

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The control of blood glucose levels in vertebrates is a fundamental problem that has long intrigued both clinical and basic researchers. Much is known of the circulating hormones (e.g., insulin, glucagon, and glucocorticoid) that regulate the metabolism of carbohydrate and fat. The structures of these hormones have been determined, their receptors identified, and in general terms, their modes of action are understood. Likewise, considerable information has accumulated concerning the hormone-regulated enzymes involved in the control of gluconeogenesis and the synthesis and mobilization of glycogen and triglycerides. The amino acid sequences of many of these enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK), glucokinase (GK), tyrosine aminotransferase (TAT), hormone-sensitive lipase (HSL), and glycerol-3-phosphate dehydrogenase (GPDH) are known, and their encoding genes have been cloned.

One issue central to the problem of energy homeostasis concerns the differentiation of liver and adipose tissues, respectively, as the principal sites of carbohydrate and fat metabolism. How does the liver become specified as the organ primarily responsible for gluconeogenesis and the storage and mobilization of glycogen? How does adipose tissue become the storage depot of triglycerides? Both tissues provide the major circulating physiological fuels (glucose, free fatty acids, and ketones). What mechanisms facilitate the elevated levels of expression of gluconeogenic and lipogenic enzymes in these respective tissues? How do these enzymatic activities become subject to regulation by the hormones that control blood sugar levels?

The answers to some of these questions are beginning to emerge. For example, the abundance of PEPCK, GK, and TAT enzymatic activities in liver is correlated with relative increases in their mRNA levels. Likewise, GPDH and HSL transcripts accumulate selectively in differentiated adipocytes. What causes the genes encoding these mRNAs to be expressed at elevated levels in the appropriate cell types? Moreover, what distinguishes the PEPCK, GK, TAT, and glucose-6-phosphatase (G-6-P) genes, whose expression in liver cells is regulated hormonally, from other genes expressed in hepatocytes that are not responsive to regulation by the circulating factors that modulate energy homeostasis?

In this communication we speculate that regulation of energy balance may be channeled, at least in part, through a recently discovered gene regulatory protein, termed CCAAT/enhancer-binding protein (C/EBP). The

reasoning behind this speculation will follow a brief description of the properties of C/EBP.

The protein was identified originally as a heat-stable, sequence-specific DNA-binding activity present in soluble extracts of rat liver nuclei (Graves et al. 1986; Johnson et al. 1987). Purified C/EBP selectively recognized CCAAT homologies and enhancer core sequences, implying that it may be a transcriptional regulatory protein. Recent studies indicate that the optimal C/EBP-binding site consists of directly abutted, dyad half-sites bearing the pentanucleotide sequence 5'-GCAAT-3' (Vinson et al. 1989). Early speculations that the protein may specify two distinct DNA-binding activities have not been borne out. Instead, C/EBP appears, at least when tested *in vitro*, to be capable of recognizing sites on DNA that diverge significantly from its optimal, dyad-symmetric recognition site. The region of the protein necessary for recognition of all sites tested thus far is a 60-amino-acid segment located close to the carboxyl terminus of the protein (Landschulz et al. 1988a, 1989).

Biochemical studies of C/EBP eventually led to the generation of specific antibodies and isolation of a molecular clone of its encoding DNA (Landschulz et al. 1988b). These reagents facilitated studies of the tissue distribution and developmental expression pattern of C/EBP (Birkenmeier et al. 1989). The latter study offered the initial indication that the physiological function of C/EBP might be related to energy metabolism. C/EBP mRNA was found at high levels in adipose, liver, and placental tissues and at lower levels in lung and small intestine. One property shared by these tissues is the capacity to metabolize lipids, cholesterol, and other sterols at exceptionally high rates (see Birkenmeier et al. 1989). Furthermore, aside from lung, each of these tissues can be considered as part of the network of organs that facilitates the uptake, metabolism, and storage of physiological fuels. For example, adipocytes and hepatocytes store and mobilize triglycerides and glycogen, respectively. The placenta plays a vital role in fetal nutrition, and the gut is responsible for the uptake of dietary fat and carbohydrate.

The availability of a cloned copy of the gene encoding C/EBP made possible a second series of experiments, the results of which also point to a relationship between C/EBP and energy homeostasis. An expression vector that allows expression of C/EBP in mammalian cells has been found to be capable of activating transcription of liver- and adipose-specific genes that carry its binding

site. To date, the promoters of four genes involved in energy metabolism have been found to be *trans*-activated by C/EBP. These include the genes that encode serum albumin (Friedman et al. 1989), 422/aP2 protein and stearoyl acyl-CoA desaturase-1 (SCD1) (Christy et al. 1989), and the insulin-responsive glucose transporter (GT2) (Kaestner et al. 1989). It is notable that both albumin and 422/aP2 protein bind and transport long-chain fatty acids. SCD1, which serves to desaturate fatty acids for transport and storage, is expressed in adipose tissue and liver, which are the major sites of C/EBP expression. GT2 gates the entry of glucose into adipocytes for lipogenesis and is regulated by insulin, the hormone that promotes global energy storage. Moreover, two of these adipose-specific genes (422/aP2 and SCD1) are activated not only by C/EBP (Christy et al. 1989) but also by cAMP (Ntambi et al. 1988; Yang et al. 1989), the key pleiotropic mediator of energy metabolism in adipose tissue and liver. Recent findings indicate that the effect of cAMP on the transcription of the 422/aP2 and SCD1 genes is mediated through C/EBP (M.D. Lane and colleagues, unpubl.). Inductive effects of cAMP have also been observed in the C/EBP-mediated activation of the serum albumin promoter (A. Friedman and S. McKnight, unpubl.).

The third finding consistent with a relationship between C/EBP and the control of energy homeostasis came from studies of C/EBP mRNA expression during rat and mouse development (Birkenmeier et al. 1989). The concentration of C/EBP increased dramatically in both liver and intestinal tissue shortly before birth—the time at which blood sugar levels must first become regulated independently of maternal influence. This is the time at which PEPCK enzymatic activity first appears in the liver and when gluconeogenesis begins. Moreover, birth is also the time at which the gut must first digest and absorb fat and carbohydrate from the mother's milk. Because a coordinate increase in C/EBP mRNA concentration was observed in both liver and gut, the possibility arose that expression of the gene encoding C/EBP may be responding to humoral regulators that were preparing the fetus for the transition to the state of independent energy balance.

The most recently discovered link between C/EBP and energy metabolism has come from an examination of two overlapping deletion mutations near the albino locus on chromosome 7 of the mouse (for review, see Gluecksohn-Waelsch 1979). Mice homozygous for these 'perinatally lethal albino' deletions die shortly after birth. Although the exact cause of death has not been established, extensive studies have shown that these lethal albino deletions lead to severe defects in liver function. The defects include reduction in the levels of gluconeogenic enzymes such as PEPCK, TAT, and G-6-P, as well as reductions in the synthesis and secretion of serum proteins such as albumin, α -fetoprotein, and transferrin. Not surprisingly, newborn homozygotes are severely hypoglycemic (Erickson et al. 1968; Gluecksohn-Waelsch 1986). The multiple effects of different deletions provided the necessary criteria for comple-

mentation tests that resulted in a tentative complementation map of the overlapping deletions at and around the albino locus, limited proximally by the marker taupe, 2 cM from the albino locus, and distally by shaker-1, 4 cM from the albino locus. The structural genes encoding PEPCK, TAT, G-6-P, albumin, α -feto-protein, and transferrin have been shown to map to chromosomes other than that carrying the lethal albino deletions (Cori et al. 1981, 1983). These and other observations have been interpreted to indicate that the perinatal lethal albino deletions uncover the function of a cell-type-specific regulatory gene, or a closely linked set of genes (for review, see Gluecksohn-Waelsch 1987). This hypothetical gene has been termed hepatocyte-specific developmental regulator (*hsdr*).

Although *hsdr* and *C/ebp* are both located on mouse chromosome 7, the two genes map ~30 cM apart from one another (Miller et al. 1974; Birkenmeier et al. 1989). However, knowing that the phenocritical period of the hypothetical *hsdr* gene product(s) (HSDR) coincided with a striking period of C/EBP mRNA induction, we tested whether HSDR may influence the expression of C/EBP. Livers were dissected from newborn animals that were homozygous (c^{3H}/c^{3H}) for the lethal albino deletion, as well as from control littermates (c^{3H}/c^{ch} and c^{ch}/c^{ch}). As shown in the left and middle panels of Figure 1, C/EBP mRNA concentration, relative to an internal β -tubulin control, is substantially reduced in the homozygous c^{3H} animals.

If the reduced level of C/EBP mRNA in the livers of lethal albino homozygous mice accounts, in any way, for the physiological effects observed in these animals, one might expect that the affected genes would be regulated by C/EBP. Evidence supporting this expectation has begun to emerge. C/EBP binds to the promoter of the serum albumin gene (Lichsteiner et al. 1987; Costa et al. 1988) and, as mentioned previously, acts as a potent *trans*-activator of the albumin promoter in cultured HEPG2 cells (Friedman et al. 1989). C/EBP also binds to the promoters of the transferrin gene (Brunel et al. 1988) and the PEPCK gene (M. Trus and L. Reshef, unpubl.; R. Hanson, unpubl.). Indeed, recent studies on the PEPCK gene indicate that C/EBP binds to the *cis*-regulatory element that mediates response to cAMP and that a C/EBP expression vector is capable of *trans*-activating PEPCK gene expression in cultured cells that otherwise lack C/EBP (R. Hanson, unpubl.). These observations raise the possibility that the hypoglycemic state typical of the perinatally lethal mice results, at least in part, from a reduced level of C/EBP.

If C/EBP does play a central role in the maintenance of energy balance, why is it expressed in the lung? It has been speculated that C/EBP may be needed by secretory cells that line the alveolar sacs of the lung (Birkenmeier et al. 1989). These cells synthesize large amounts of phospholipids, which combine with certain specialized proteins to form the surface-tension-reducing component (surfactant) of the bronchial epithelium (King 1982; Goerke and Clements 1986). Even if such speculation proves to be correct, it is difficult to reconcile this

Figure 1. Primer extension mapping of C/EBP and β -tubulin mRNAs in liver and lung tissue from lethal albino mice and control littermates. Total RNA was purified from liver and lung tissue that had been dissected from lethal albino homozygous mice (c^{3H}) and control littermates (wt) 2 hr after birth. For each primer extension assay, 10 μ g of RNA was hybridized with 5 ng of 32 P-labeled, synthetic primer DNA. The nucleotide sequence of the C/EBP primer was 5'-CCGCCGCCACCCAGTACCC-CAACTCGC-3'. The nucleotide sequence of the β -tubulin primer was 5'-AGCCCAAATCATCATGCGGAG-3'. Annealed primer was extended using AMV reverse transcriptase (for a detailed description of conditions used for primer extension, see Graves et al. 1986). Primer extension products were sized on an 8 M urea–8% polyacrylamide sequencing gel, which was used to generate the autoradiographic exposures shown. (Left) Assays of β -tubulin mRNA derived from liver tissue; (middle) assays of C/EBP mRNA derived from liver tissue; (right) assays of C/EBP mRNA derived from lung tissue. Full-length primer extension products are designated by arrows at left (β -tubulin) and right (C/EBP).

bronchial function of C/EBP with its proposed role in energy homeostasis. A potential solution to this enigma emerged from a comparison between the effect of the lethal albino deletion on C/EBP mRNA expression in the lungs of c^{3H} homozygous animals and that in their normal littermates. As shown in Figure 1 (right panel),

C/EBP mRNA concentration was not affected in the lungs of homozygous deletion mutants, whereas, hepatic C/EBP mRNA levels of the same animals were affected. The cell type-specific effects of lethal albino deletions have been observed in studies of the expression of other genes, including the mouse metallothionein-1 gene (DeFranco et al. 1987). The analogous observations presented here uncouple the action of C/EBP in lung from its role in hepatic tissue. These observations indicate that C/EBP function will encompass a regulatory circuit that is more global than energy balance, perhaps pertaining, in the most general sense, to lipid synthesis.

The interpretations offered in this communication raise many obvious questions. Does C/EBP constitute a direct regulator of each gene that is underexpressed in the livers of animals lacking *hsdr* function? Do the liver genes that are expressed at normal levels in the absence of HSDR not bind C/EBP? Do the effects on the expression of affected genes result solely from a reduction in the level of C/EBP mRNA, or is the reduction in C/EBP mRNA symptomatic of a more widespread set of defects caused by the dysfunction of *hsdr*? That is, at what level does the *hsdr* gene product regulate C/EBP? Might it be a transcription factor that controls expression from the *C/ebp* structural gene, or is it some part of the signal transduction system central to the action of glucagon, insulin, or glucocorticoids? The latter possibility is particularly attractive in light of studies that have shown that *hsdr* regulates hormonally inducible, but not constitutive, gene expression in the liver (Donner et al. 1988). Moreover, if C/EBP is a central player in the physiological circuits that have evolved to regulate the levels of circulating fuels (e.g., blood glucose and free fatty acids), how does it respond to the relevant hormones? Is C/EBP action modified by cAMP, as would be expected if it is the target of glucagon action? Might its activity also be regulated by either the insulin receptor tyrosine kinase or an isoform of protein kinase C, as would be expected if it is the target of insulin action? If C/EBP induces the expression of enzymes such as G-6-P and PEPCK, which generate circulating fuels, then what is its role in the expression of genes that encode glycolytic enzymes? Finally, how are its modes of action in one tissue, such as liver, related to other tissues, such as adipose or intestine? In a fasting animal, does C/EBP orchestrate a program to mobilize triglycerides in one tissue (adipose) and glycogen in another tissue (liver) in a concerted manner?

The issues outlined in the preceding paragraph are almost entirely unresolved. Although its role in energy metabolism may be one of the major tasks of C/EBP, it is no doubt responsible for the regulation of a broader range of events. Also, its relationship to *hsdr* remains to be analyzed more fully. Regardless of these uncertainties, the molecular reagents necessary for conducting precise experiments on the physiological role of C/EBP are now available, and we hope that the implementation of these reagents will lead to a better understanding of the role of this protein in mammalian growth and development.

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