Commentary

ADD-1 provides major new insight into the mechanism of insulin action

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We evolved to live in a world in which food supplies are unpredictable in availability and often insufficient to meet immediate needs. Consequently, it is critical to be able to switch between the distinct metabolic programs that are appropriate to either the fasted or the fed state. Although many endocrine systems are involved in this transition, insulin is a preeminent hormonal mediator of this nutritional switch. Insulin levels rise postprandially to mediate anabolism and energy storage and fall between meals to permit the release of adipose energy stores and the switch from glucose use to production. The relevant actions of insulin take place in adipose tissue, muscle, and liver, where insulin receptor signaling experts tissue-specific effects on an array of metabolic pathways that impact on carbohydrate, lipid, and protein metabolism. The cellular mechanism of insulin action has been a topic of major interest for many years. The greatest emphasis has been on the early steps of signal transduction, involving the insulin receptor substrate (IRS) proteins, which when tyrosine phosphorylated by the insulin receptor kinase serve as docking proteins for assembly of signaling complexes for downstream pathways such as ras-mitogen-activated protein kinase, phosphatidylinositol 3-kinase, and others (1). Many classic actions of insulin (e.g., glucose transport and glycogen synthesis) occur very rapidly and require the mediation of rapid changes in the state of protein phosphorylation or dephosphorylation. It is established that, alongside these rapid nongenomic effects, changes in gene expression play critical roles in insulin action in adipose cells, liver, and other tissues (2).

Although insulin-responsive DNA elements have been identified in the promoters of several insulin-regulated genes, the identity of transcription factors responsible for mediating key insulin actions on gene expression has been, until recently, obscure. Two recent papers in PNAS (3, 4) provide important new information on the identity of an insulin-responsive transcription factor that appears to be linked to regulation of hepatic glucose and lipid metabolism. This transcription factor, known alternatively as ADD-1 (adipocyte determination and differentiation factor-1) (5) and SREBP-1c (sterol regulatory element binding protein-1c) (6), previously was shown to regulate the expression of key genes of fatty acid and triglyceride metabolism in fibroblasts, adipocytes, and the livers of transgenic mice (7, 8). Taken together with recent data that ADD-1/SREBP-1c might mediate insulin effects on gene expression in adipose cells (9), ADD-1/SREBP-1c now appears to be a strong candidate to be a general mediator of the action of insulin to regulate metabolism via effects on gene expression (Fig. 1). The details of this pathway promise to be important for future studies of insulin action and diabetes pathogenesis.

ADD-1/SREBP-1 is a transcription factor that was cloned independently by two groups through unrelated approaches, using distinct experimental systems. The group of Spiegelman (5) identified ADD1 as a basic helix–loop–helix transcription factor expressed in adipocytes and regulated during both determination and differentiation of cultured adipocyte lines (5). The initial paper defined an ability to activate transcription through an element in the flanking region of the fatty acid synthase (FAS) gene, which is expressed in a differentiation-dependent manner in adipocytes, and in response to feeding in adipose tissue in vivo. One element of research on this factor has examined its ability to influence adipogenesis, cooperating with peroxisome proliferator-activated receptor-γ by increasing production of a lipid ligand able to activate this transcription factor (10) or increasing its expression (11). A second area of investigation of more immediate relevance to this discussion involves a possible role for ADD1/SREBP-1c in mediating the transcriptional effect of insulin in adipocytes. Kim et al. (9) demonstrated that mRNA encoding ADD1/SREBP-1c was suppressed by fasting and restored by feeding in adipose tissue of the mouse, and that this closely paralleled the expression of two adipocyte genes that are likewise regulated by nutrition, FAS, and leptin. To complete the mechanistic loop, those authors made the critical observation that insulin could mimic these effects when added to adipocytes in culture, and that ADD1/SREBP-1c could transactivate both FAS and leptin promoters (9). Insulin’s stimulatory effect on the FAS promoter was mapped to an E-box motif, contained within a sequence previously identified as the major insulin response element of this gene. Thus, it was established that ADD-1/SREBP-1 was a transcription factor involved both in adipogenesis and in the regulation of insulin-responsive genes in adipose cells.

Brown, Goldstein, and colleagues (6) identified SREBP-1 and -2 through a completely different line of investigation. These workers purified and cloned SREBPs from HELA cells as a family of three proteins that bind to sterol regulatory elements and activate transcription of several genes involved in cholesterol homeostasis (6). Depletion of cholesterol stimulates proteolytic release of the transcriptionally active amino terminus of these molecules from a position within the endoplasmic reticulum membrane, permitting movement to the nucleus (12, 13). Three SREBPs are produced by two genes, with SREBP-1a and -1c transcribed from a single gene by alternative promoter use, and SREBP-2 the product of a distinct gene, with 50% sequence homology to the SREBP-1 isoforms. A number of lines of evidence suggest that SREBP-2 is more associated with cholesterol metabolism, and SREBP-1 is more associated with control of genes involved in fatty acid and other lipids. When truncated dominant positive forms of SREBP-1a and -1c were transgenically expressed in liver, massive lipid overproduction ensued, because of increased expression of lipogenic enzyme pathways, with increased effectiveness of SREBP-1a vs. -1c (8). Hints

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that these effects might be related to the mechanism of action of insulin, an important lipogenic hormone in liver, came from two observations. First, the SREBP transgenics (with constitutive overexpression of the activated isoform) did not show a fall in lipogenic enzyme gene expression with fasting. Second, insulin was directly shown to stimulate SREBP-1c mRNA in isolated hepatocytes (14).

The two recent reports in PNAS extend these findings considerably. Foretz et al. (3) have used adenoviral vectors expressing either a dominant negative SREBP-1c or a dominant positive version of this protein (3). In the isolated hepatocyte model, they show that dominant negative SREBP-1c blocks the ability of insulin to induce the glucokinase gene, and a dominant positive version stimulates glucokinase gene expression in the absence of insulin, which is otherwise necessary for expression of this gene. Shimomura et al. (4) bring this finding to the in vivo level, by demonstrating that SREBP-1c (but not SREBP-1a) mRNA is specifically diminished in the liver of mice made diabetic with the β cell toxin streptozotocin and is rapidly induced after insulin therapy (4). This specific effect was reproduced in vitro with isolated hepatocytes. Taken together, ADD-1/SREBP-1c appears to be a transcription factor poised to mediate many of the actions of insulin on expression of genes involved in both lipid and carbohydrate metabolism in two key tissues, fat and liver. Given the limited success of previous efforts to identify molecular mediators for the key transcriptional actions of insulin, these new data are important and greatly welcomed.

Many critical questions remain unanswered, however. First, how general is the role of ADD-1/SREBP-1c in mediating the effect of insulin on gene expression? It will be necessary to determine the effects of ADD-1/SREBP-1c on expression of a larger number of insulin-dependent genes in adipocytes and hepatocytes and to determine the role of ADD-1/SREBP-1 in other important tissues, such as muscle. Might this factor be involved in both positive and negative regulation, or is repression of key genes such as phosphoenolpyruvate carboxykinase (PEPCK) mediated by distinct factors? What are the mechanisms by which insulin regulates ADD-1/SREBP-1? It certainly appears that increased gene expression is a key mechanism. Studies of the ADD-1/SREBP-1 promoter will be needed to define the mechanism, and eventually the signaling pathways, used by insulin in regulation of this gene. A possible effect of insulin to induce serine phosphorylation of ADD-1/SREBP-1 was mentioned in an earlier paper (9), but data on this point are lacking, and this step could be important. Finally, given the elegant work demonstrating the role for proteolytic processing of SREBP-2 in the regulation of sterol metabolism (13), it will be critical to determine whether insulin plays a previously unappreciated role in activating the processing of SREBP-1c in the liver. Whatever the answers to these questions, ADD-1/SREBP-1c is well positioned to be at the intersection of regulatory pathways for carbohydrate and lipid metabolism in response to nutrition and insulin, and possibly other hormonal factors.

A final issue relates to the possible role of ADD-1/SREBP-1c in disease. If this transcription factor is indeed a central mediator of the genomic actions of insulin on both carbohydrate and lipid metabolism in both adipose and liver cells (as well as other cells to be determined), then it is in a pivotal position to influence insulin action and promote disease by altering insulin responsive pathways. This notion suggests that the gene itself, as well as factors apart from insulin that regulate it, should be examined in diseases such as type II diabetes, obesity, and other states of insulin resistance. For all of these reasons, it seems certain that ADD-1/SREBP-1c will attract considerable attention from investigators in a number of related fields for years to come.