0163-769X/03/$20.00/0
Printed in U.S.A.

Endocrine Reviews 24(1):78–90

doi: 10.1210/er.2002-0012

Peroxisome Proliferator-Activated Receptor-γ Coactivator 1α (PGC-1α): Transcriptional Coactivator and Metabolic Regulator

PERE PUIGSERVER AND BRUCE M. SPIEGELMAN

Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115

Investigations of biological programs that are controlled by gene transcription have mainly studied the regulation of transcription factors. However, there are examples in which the primary focus of biological regulation is at the level of a transcriptional coactivator. We have reviewed here the molecular mechanisms and biological programs controlled by the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). Key cellular signals that control energy and nutrient homeostasis, such as cAMP and cytokine pathways, strongly activate PGC-1α. Once PGC-1α is activated, it powerfully induces and coordinates gene expression that stimulates mitochondrial oxidative metabolism in brown fat, fiber-type switching in skeletal muscle, and multiple aspects of the fasted response in liver. The regulation of these metabolic and cell fate decisions by PGC-1α is achieved through specific interaction with a variety of transcription factors such as nuclear hormone receptors, nuclear respiratory factors, and muscle-specific transcription factors. PGC-1α therefore constitutes one of the first and clearest examples in which biological programs are chiefly regulated by a transcriptional coactivator in response to environmental stimuli. Finally, PGC-1α's control of energy homeostasis suggests that it could be a target for antiobesity or diabetes drugs. (*Endocrine Reviews* 24: 78–90, 2003)

I. Introduction

GENE TRANSCRIPTION IS now understood to involve several distinct protein complexes that are necessary to get proper regulation in space and time. The transcriptional machinery must function at the correct time and chromosomal location to unwind compacted chromatin, recruit RNA polymerase and related factors, and initiate RNA synthesis. The key components can be organized into several categories. First, transcription factors generally refer to proteins that bind to DNA in a sequence-specific manner, typically as hetero- or homodimers. Most commonly, this occurs in the region 5′ to the transcription start site. It was previously thought that transcription factors themselves were directly involved in recruiting RNA polymerase II or unwinding DNA, but it is now understood that this function is largely carried out by other proteins. Second, the region around the start of transcription binds the general transcription apparatus, a complex that functions to correctly orient RNA polymerase II at the specific site for initiation of transcription. This complex, involving many proteins, usually contains TATA binding protein, although proper initiation can also occur without this factor. Finally, mediating the functional connection between transcription factors and the general transcription apparatus are the coactivators. Coactivator refers to a protein or protein complex that increases the rate of transcription by interacting with transcription factors but does not itself bind to DNA in a sequence-specific manner. The binding of coactivators to transcription factors can also be regulated, for example, by the binding of ligands to nuclear hormone receptors.

Coactivators usually exist and function as multiprotein complexes. These complexes contain individual proteins that mediate docking on transcription factors and others that mediate functions necessary for transcription itself. These include modifications of histones by acetyltransferase activity, modification of histones by phosphorylation or methylation, and the unwinding and remodeling of chromatin in an ATP-dependent fashion. It is also highly likely that addi-

Abbreviations: AF, Activation function; BAT, brown adipose tissue; CBP, CREB binding protein; CREB, cAMP response element binding protein; ER, estrogen receptor; Glut-4, insulin-sensitive glucose transporter; GR, glucocorticoid receptor; HAT, histone acetyl transferase; HNF-4α, hepatocyte nuclear factor-4α; MEF2, myocyte enhancer factor-2; mtTFA, mitochondrial transcription factor A; NRF, nuclear respiratory factor; PEPCK, phosphoenolpyruvate carboxykinase; PGC, PPAR-γ coactivator 1; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RMM, RNA-binding motif; RS, serine-arginine-rich; SRC-1, steroid receptor coactivator-1; TR, thyroid receptor; TRAP, TR-associated protein; UCP, uncoupling protein; WAT, white adipose tissue.

tional enzymatic activities associated with coactivator complexes will be discovered.

The last 20 yr have seen tremendous progress in our understanding of the role of transcriptional control in almost every area of biology—development, physiology, and disease. In the vast majority of cases described to date, a key regulatory step is an alteration in the amount or activity of the transcription factor. However, recent work has shown that biological regulation can also be achieved through modulation of other components of the transcriptional apparatus. The first example of biological regulation at the coactivator level was the demonstration of the key function of a B cell-selective coactivator of the Oct family of transcription factors, Oca-B. Our studies of the regulation of the adipose cell lineage, especially in the divergence of the white *vs.* brown fat pathways, led us to discover a coactivator with an amazing degree of regulation in different tissues and physiological states. Peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC-1α), the subject of this review, is involved in multiple biological responses related to energy homeostasis, thermal regulation, and glucose metabolism.

II. PGC-1α Regulates Biological Programs Linked to Energy Homeostasis

A. PGC-1α and adaptive thermogenesis

1. Concepts and tissues involved in adaptive thermogenesis. Energy balance is defined by two components: energy intake and energy expenditure. Chronic perturbations in these two components can lead to an increase or decrease in body weight. In homeotherms, energy expenditure is the result of basal metabolism, physical activity, and adaptive thermogenesis (also known as facultative thermogenesis; see Ref. 1). Adaptive thermogenesis refers to changes in heat dissipation in response to environmental temperatures, nutritional status, or infection. In each of these challenges, physiological mechanisms are activated to increase heat production in different tissues, particularly brown fat and muscle (2, 3).

Most adaptive thermogenesis in small mammals takes place in brown adipose tissue (BAT). BAT is morphologically and metabolically different from white adipose tissue (WAT) and plays an opposite physiological function: dissipation *vs.* storage of energy. Brown adipocytes contain multiple small droplets of triglycerides and a high number of mitochondria. In addition, their mitochondria contain a specific uncoupling protein-1 (UCP-1), which is expressed only in brown adipocytes. Genetic studies from mice lacking UCP-1 indicate that it is an essential component for cold-induced thermogenesis; however, it seems to play a smaller role for diet-induced thermogenesis (4). Adaptive thermogenesis in BAT is achieved through two main inputs: adrenergic stimulation (5, 6) and thyroid hormone action (7, 8).

Another tissue involved in adaptive thermogenesis is skeletal muscle, particularly in large adult mammals in which there is relatively little brown fat (9, 10). Skeletal muscle represents about 40% of the total body mass and has high oxidative metabolism linked to a high mitochondrial number (11).

Once it was appreciated that the molecular mechanism for brown fat thermogenesis is through uncoupling respiration depending on UCP-1, it was reasonable to speculate that other tissues, such as muscle or liver, might use similar systems to generate heat. By searching for genes similar to UCP-1 in tissues other than brown fat, several groups cloned UCP-2 (12, 13) and UCP-3 (14–16). Genetic loss-of-function mice have been created that lack UCP-2 or UCP-3 (17). These data support the idea that these proteins participate in uncoupling respiration in mitochondria. However, these mice did not have any alteration in thermogenic function or systemic energy balance. Rather, UCP-2 has been shown to be important in free radical production, defense against infection (18), and insulin secretion in the pancreas (19).

2. PGC-1α regulates components of adaptive thermogenesis and mitochondrial biogenesis.

a. Identification of PGC-1α. Although some of the effectors for adaptive thermogenesis, such as UCP-1, have been extensively studied, relatively little is known about the transcriptional regulation of this process. One useful approach to this problem has been the analysis of the specific brown fat-specific enhancer of UCP-1. UCP-1 gene expression is highly cold inducible through the activation of the sympathetic nervous system and is mediated by β-adrenoreceptors and cAMP (20, 21). In addition, several activated nuclear hormone receptors play an important role in the differentiation of brown fat cells and in UCP-1 gene expression, including thyroid hormone receptor (TR), retinoic acid receptor (RAR; Ref. 22), and PPARs (PPARα, PPARγ; Refs. 23 and 24). The potential involvement of PPARγ in this regulation is particularly interesting because of its key role in white fat-specific gene expression and differentiation. Activation of PPARγ by synthetic ligands, the antidiabetic thiazolidinediones, has been shown to promote the differentiation of brown fat precursor cells and to cause a hypertrophy of BAT when given to rats (25, 26). Furthermore, PPARγ knockout animals lack brown adipose tissue, indicating that it is required for the formation of this tissue, as well as white fat (27, 28). Although these data strongly support the importance of PPARγ in brown fat differentiation, it is clear that the same factor alone cannot determine whether adipocyte cells are white (energy storage) or brown (energy dissipation). These data suggested to us the possibility that a specific cofactor might augment and alter PPARγ function in brown fat cells, resulting in activation of the thermogenic genes that characterize this cell type. Based on this hypothesis, we cloned a PPARγ-interacting protein that is expressed preferentially in brown fat compared with white fat, in which endogenous levels are very low. We called this factor PGC-1α (29).

b. Adrenergic regulation of PGC-1α in brown fat and muscle. The function of the β-adrenergic receptors in brown fat and muscle is critical for the response of these tissues to cold and alterations in diet. Physiologically, this stimulation is mediated through the sympathetic nervous system that innervates the brown adipocytes. PGC-1α is strongly induced at the transcriptional level in brown fat and skeletal muscle when mice are exposed to the cold. Consistent with these *in vivo* data, PGC-1 mRNA is induced in a brown fat cell line treated with a β-adrenergic receptor agonist, isoproterenol (29). The effect is mediated through the β3-adrenergic pathway, in that

knockout animals for this receptor lack the cold induction of PGC-1α in brown fat (30). In addition, injection of specific β3-agonists can specifically induce PGC-1α in brown fat (31). Although much less is known about PGC-1β, a close homolog of PGC-1α, it is expressed in brown fat but is not induced upon cold exposure (32).

Interestingly, the adrenergic induction of UCP-1 has been studied in detail, but the transcription factors involved remained unknown. As stated above, PGC-1α strongly coactivates several nuclear receptors that bind to the UCP-1 enhancer. Thus, a likely model is that a major portion of the cAMP effect is mediated via the induction of PGC-1α and subsequent interaction of this factor with nuclear hormone receptors PPARγ, PPARα, RAR, and TR. In interesting genetic studies, Kozak (33) has found quantitative trait loci in mice linked to specific chromosomes that regulate both PGC-1α and UCP-1 expression and could account for the appearance of brown fat in white fat depots.

c. PGC- 1α and the adipocyte cell fate decision. A key question is whether PGC-1α is the major switch in the decision to become either white or brown fat cells. Although it has been proven that PPARγ is necessary and sufficient to induce white fat differentiation, it is necessary but not sufficient to induce brown fat differentiation (27, 28). Several pieces of data suggested that PGC-1α is likely to be involved in this decision (29). First, PGC-1α is the only protein thus far described that can powerfully activate the UCP-1 enhancer in non-BAT cell lines. Second, when introduced into white fat cells, PGC-1α induces endogenous UCP-1 gene expression and mitochondrial biogenesis, two decisive features of brown fat. Finally, PGC-1α is a downstream target of the adrenergic signaling, which is the key signaling mediator of brown fat differentiation in cell cultures and after cold exposure. Although these correlations are consistent with PGC-1α playing a key role in the adipocyte cell fate decision (Fig. 1), experiments to date in culture have not expressed PGC-1α levels comparable to those *in vivo*, and the resulting UCP-1 gene expression is also far below the *in vivo* levels. Hence, a more definitive proof of the requirement and sufficiency of PGC-1α as the key switch for the decision between

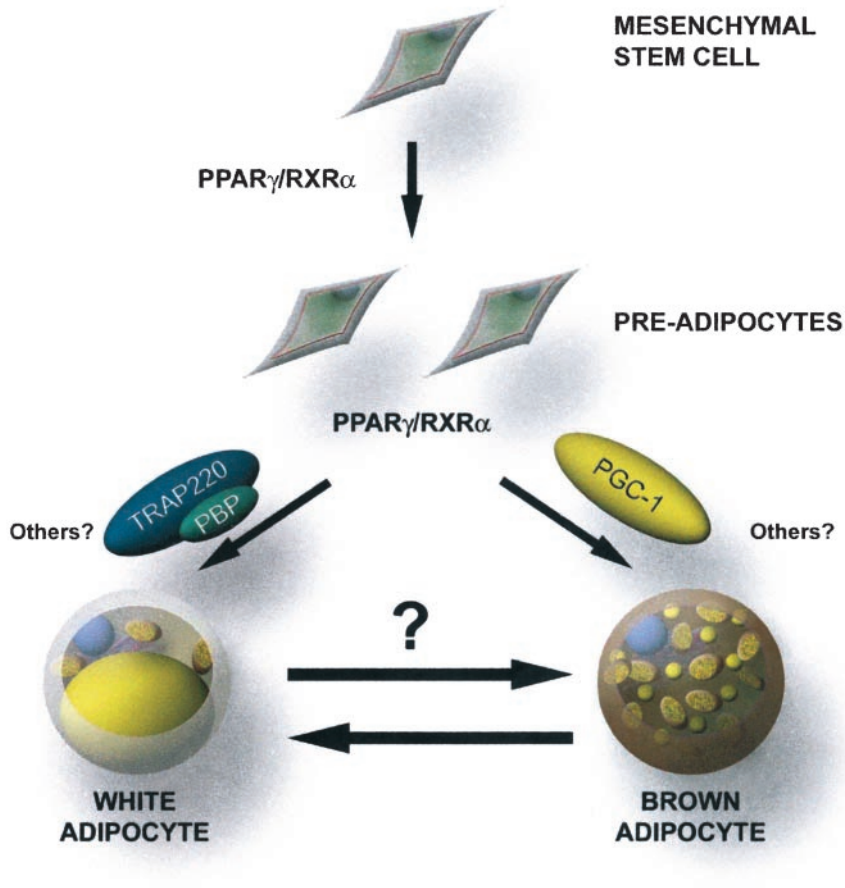

FIG. 1. Transcriptional control of adipocyte cell fate. The activation of PPARγ/RXRα is a key function in the differentiation of preadipocytes. Preadipocytes can potentially differentiate into two different types of adipocytes: white and brown. Activation of PPARγ/RXRα is required for the differentiation of both types of adipocytes. A key switch in this bifurcation is the presence of particular PPARγ coactivators. For instance, it is known that the coactivator TRAP220/PPARγ binding protein (PBP), part of the TRAP/vitamin-D receptor interacting proteins complex, is required for white adipocyte differentiation (76). On the other hand, the utilization of PGC-1α coactivator by PPARγ can activate specific gene expression associated with the conversion of preadipocytes to brown adipocytes. Whether there is a conversion between brown adipocytes and white adipocytes is unknown.

brown and white fat cell lineages will require the manipulation of PGC-1α levels in mouse models.

d. PGC-1α regulates respiration, mitochondrial biogenesis, and gene expression. Enhanced mitochondrial biogenesis is an important component of adaptive thermogenesis, especially in brown fat and skeletal muscle, in which PGC-1α is highly expressed and inducible by cold or adrenergic stimuli. Exposure to cold temperatures induces mitochondrial proliferation and increased uncoupling of respiration. The role of thyroid hormone in stimulating mitochondrial biogenesis and in the induction of mitochondrial genes encoded in the nucleus is well established (34). Scarpulla and collaborators (35, 36) identified and cloned two novel transcription factors, nuclear respiratory factor (NRF)-1 and -2, that bind to the promoter region of a broad range of mitochondrial genes encoded in the cell nucleus, including β-ATP synthase, cytochrome-c, cytochrome-c-oxidase subunit IV, and mitochondrial transcription factor A (mtTFA). It is of particular interest that the NRFs turn on mtTFA, a key transcriptional activator that translocates to the mitochondria and activates mitochondrial DNA replication and transcription.

PGC-1α has a major impact on the NRF system (Fig. 2). When introduced into muscle cells, PGC-1α dramatically induces gene expression for NRF-1, NRF-2, and mtTFA. Furthermore, PGC-1α physically interacts with NRF-1 and coactivates its transcriptional activity (37). A dominant negative allele of NRF-1 completely blocks PGC-1α's ability to induce mitochondrial proliferation, indicating the central role of this transcriptional factor/coactivator pair. Thus, PGC-1α and its functions provide a plausible molecular basis for the connection between environmental/hormonal stimuli and mitochondrial biogenesis and respiration when the organism has altered energy or thermogenic requirements.

As mentioned above, uncoupling of mitochondrial respiration is an important component of energy expenditure *in vivo*. When introduced into fat cells, PGC-1α induces UCP-1 but not UCP-2 or -3 (29). In muscle cells (in which UCP-1 is not expressed), we have shown that expression of PGC-1α

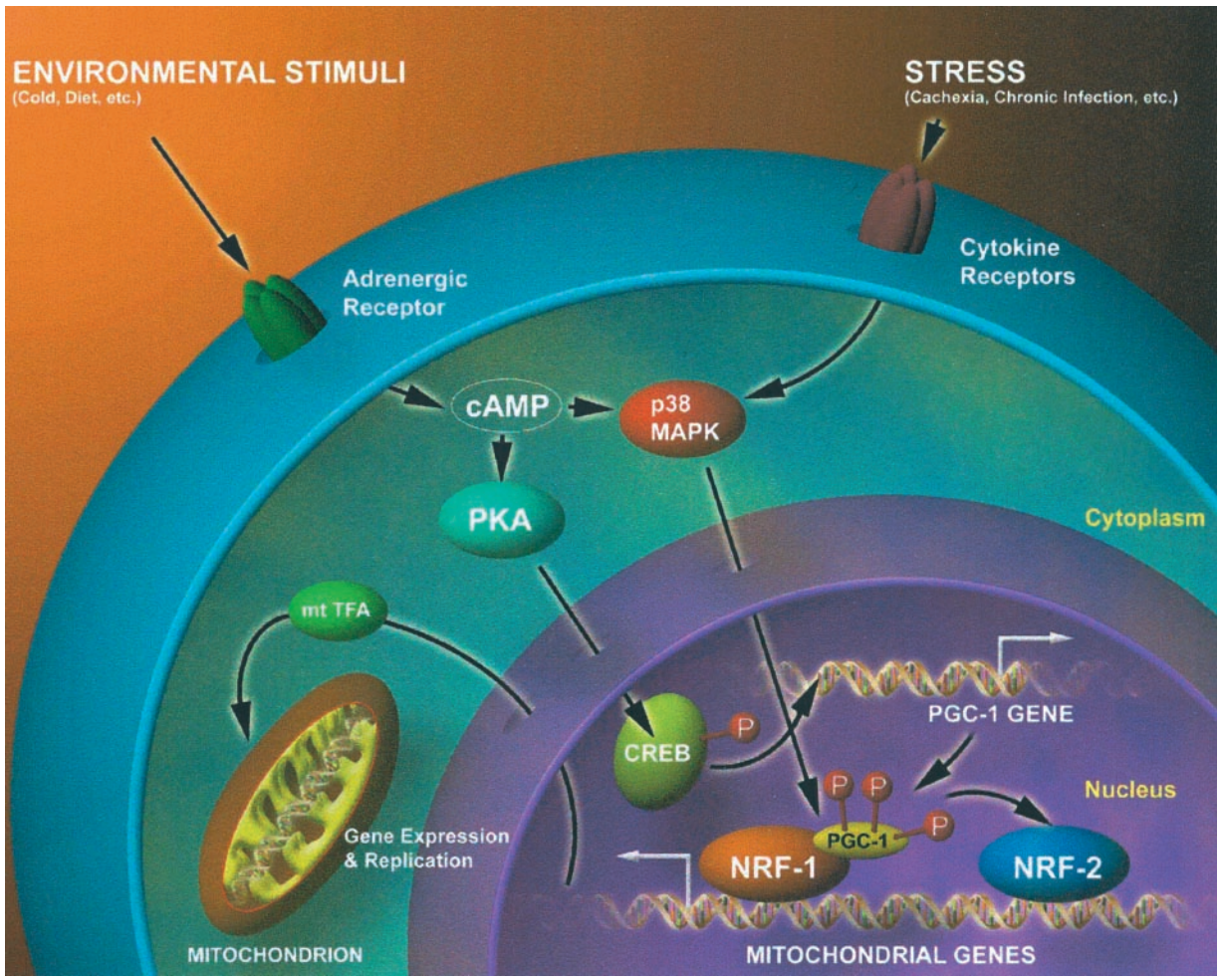

FIG. 2. Mitochondrial biogenesis and gene expression through PGC-1α. β-Adrenergic and cytokine cell surface receptors trigger signaling cascades involving the PKA and p38 MAPK pathways. PKA phosphorylates CREB transcription factor, which is involved in the induction of PGC-1α gene expression. Stimulation of p38 MAPK directly phosphorylates the PGC-1α protein, resulting in its activation and stabilization. PGC-1α activates the expression of the subunits of respiratory chain and mtTFA through the induction of the expression of NRFs and the coactivation of NRF-1-mediated transcription. mtTFA subsequently translocates into the mitochondrion and directly increases the transcription and replication of mtDNA.

also induces uncoupled respiration, but this correlated with increased expression of UCP-2 but not UCP-1 or UCP-3 (37). The transcription factor mediating this cell-specific regulation of UCP-2 in muscle cells is currently unknown. Furthermore, it is not known whether the uncoupling activity observed in either fat or muscle cells expressing PGC-1α is due to UCPs or other molecules involved in the uncoupling of respiration.

The results discussed above were performed in cultured cells, either fat or muscle. Interestingly, two different murine genetic models, one lacking the translation inhibitor of the eukaryotic initiation factor 4E binding protein (38) and the other overexpressing the forkhead transcription factor FOXC2 (39) in fat depots, display white adipose depots that begin to take on the distinctive multilocular appearance of brown adipocytes and express UCP-1. In both types of animals there was a striking correlation between the brown fat phenotype and elevated PGC-1α levels.

B. Cytokines and PGC-1α

Cachexia is a chronic state of negative energy balance and muscle wasting that is a severe complication of cancer and prolonged infection. A common feature of cachexia is an elevation in circulating cytokine levels. Elevation in inflammatory cytokines has been shown to be an important feature of cachexia induced by bacteria, HIV, a variety of cancers, and heart failure (40, 41). However, there has been little molecular mechanistic insight as to how cytokines stimulate metabolic rates. These proteins bind membrane receptors and trigger different signaling cascades involving protein kinases such as inhibitor κB kinase (42) and the stress-activated kinases p38 MAPK and c-Jun N-terminal kinase (43, 44). These enzymes phosphorylate multiple targets, including transcription factors, either in the cytoplasm or in the nucleus and regulate gene transcription.

Given the ability of PGC-1α to activate a broad program of thermogenesis, PGC-1α constitutes a potential candidate for being the target of cytokines in cachexia and energy expenditure (Fig. 2). Indeed, we have shown that cytokines such as IL-1α, IL-1β, and TNFα can activate the transcriptional activity of PGC-1α through direct phosphorylation by p38 MAPK (*Section III.E* for mechanistic details), resulting in stabilization and activation of PGC-1α protein. Cytokine or lipopolysaccharide induced activation of PGC-1α in muscle cells and muscle *in vivo*, causing increased respiration and expression of genes linked to mitochondrial uncoupling and energy expenditure (45). This study suggested a possible anticachectic benefit for the inhibition of p38 MAPK or PGC-1α activity in muscle tissue. Future studies in different cachectic murine models must address the precise involvement and/or requirement of PGC-1α in the cachectic response.

C. PGC-1α and heart development

Heart expresses relatively high levels of PGC-1α mRNA. Because this tissue is not noted for being thermogenic, it was particularly interesting to determine the function of this coactivator in the heart. PGC-1α expression is greatly increased in the developing mouse heart, immediately before the large burst of mitochondrial biogenesis and oxidative metabolism that precedes birth (46). Presumably, this burst in oxidative metabolism reflects the large requirement for ATP as the neonate heart requires more energy. When PGC-1α is introduced into cultured cardiac myocytes, increases in both mitochondrial biogenesis and respiration are observed. However, unlike what was seen in fat and muscle cells, most of this respiration was tightly coupled to ATP synthesis. Massive overexpression of PGC-1α in the hearts of mice resulted in a large increase in mitochondrial biogenesis and a dilated cardiac myopathy (46). This myopathy was thought to be due to the gross overexpression of PGC-1α.

Cardiac hypertrophy due to pressure overload in mice causes a conversion from fatty acid oxidation to glycolytic metabolism (47). PGC-1α expression decreases dramatically as fatty acid oxidation drops, suggesting that this decrease in oxidative metabolism is secondary to the loss of PGC-1α expression. It will be extremely interesting to determine whether this drop in PGC-1α levels is adaptive or maladaptive.

The specific genes regulated by PGC-1α in cardiac cells include several genes of the electron transport chain, mitochondrial biogenesis, and fatty acid β-oxidation. The regulation of carnitine *O*-palmitoyltransferase-1, a key component of β-oxidation, occurs via docking and coactivation of PGC-1α on PPARα (48). As there are multiple pathophysiological states in which cardiac mitochondrial function is altered, the PGC-1α regulatory pathway could conceivably be a target for the development of novel therapeutic strategies to improve dysfunction in certain cardiac diseases.

D. PGC-1α and fuel homeostasis: glucose uptake and gluconeogenesis

1. PGC-1α and glucose uptake. The activation of increased energy expenditure ultimately requires an increased uptake and metabolism of fuels. As mentioned above, PGC-1α has been shown to stimulate genes of fatty acid oxidation in cardiac cells, and induction is associated with an increase in fatty acid oxidation in adipocytes and in heart (46, 48). In addition, it was shown that these effects were increased when PPARα was coexpressed. Fatty acid oxidation from ^{14}C-labeled palmitate was also increased in these cells. Whether fatty acid uptake is also augmented in these cells has not been determined.

The effects of PGC-1α on glucose uptake and metabolism is of particular interest in diabetes because there are numerous studies indicating that the rates of mitochondrial oxidation can affect glucose uptake (49). Indeed, simultaneous to the effects of PGC-1α on mitochondrial respiration in skeletal muscle cells, this coactivator also induces gene expression for the insulin-sensitive glucose transporter (Glut-4) and increases glucose uptake (50). The relative insulin sensitivity of this transport process, however, does not increase. This effect on Glut-4 gene expression is partially mediated through PGC-1α binding to and coactivating the muscle transcriptional regulator MEF2 (myocyte enhancer factor-2), probably the MEF2C isoform.

2. PGC-1α and hepatic gluconeogenesis. Mammals have highly regulated systems to maintain blood glucose levels within tight limits, despite intermittent access to food. The central nervous system and red blood cells are particularly dependent on this substrate. Blood glucose levels are controlled by the hormonal modulation of glucose production and peripheral glucose uptake.

The liver is the major producer of glucose and does this through two different pathways. One is glycogenolysis, or the breakdown of glycogen that occurs in a relatively short-term fast. A second pathway, gluconeogenesis, is turned on with a medium- to long-term fast and involves *de novo* synthesis of glucose from precursors such as lactate, pyruvate, glycerol, and alanine. The rate of gluconeogenesis is controlled by the activities of three key enzymes: phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase. Hormonal modulation of these enzymes is mainly controlled at the transcriptional level with insulin, glucagon, and glucocorticoids being key. Gluconeogenesis occurs in fasting or diabetic states in which insulin is low or the liver is insulin resistant. In particular, insulin blocks the actions of the progluconeogenic hormones, glucagon and glucocorticoids. Although the promoters of genes involved in gluconeogenesis, especially PEPCK, have been studied extensively, no transcription factor has been found that is both regulated by the gluconeogenic hormones and has the ability to robustly activate the program of gluconeogenesis.

Our original studies in normal, *ad libitum*-fed mice had shown very little expression of PGC-1α mRNA in the liver. However, more recent studies illustrate that PGC-1α expression in liver is dramatically increased by fasting (51, 52). Furthermore, PGC-1α is also greatly induced in the liver of streptozotocin-injected mice, a model of type 1, insulin-deficient diabetes. It is also induced in ob/ob mice, a model of type 2 diabetes with high insulin levels but profound insulin resistance. A key role for insulin as a suppressor of PGC-1α expression in liver was shown using mice having liver-specific mutations in the insulin receptor, the LIRKO mouse. PGC-1α was highly elevated in these mice, as well.

In all of these animal models, the expression of PGC-1α correlated well with hepatic gluconeogenesis. We therefore examined the ability of gluconeogenic hormones to activate PGC-1α expression in primary hepatocytes. Glucagon, acting via cAMP, and glucocorticoids are the major positive factors activating the genes of gluconeogenesis in liver. Treatment of hepatocytes with 8-bromo-cAMP, a cell-permeable analog of cAMP, induced mRNA expression of PEPCK and glucose-6-phosphatase, key enzymes of gluconeogenesis. Dexamethasone, a synthetic glucocorticoid, increased PGC-1α gene expression only slightly but markedly synergized with 8-bromo-cAMP in the induction of PGC-1α mRNA (51).

These correlative studies led us to directly test a direct effect for PGC-1α in the activation of gluconeogenesis (Fig. 3). Primary hepatocytes were infected with adenoviruses encoding for the expression of either green fluorescent protein or PGC-1α. Remarkably, PGC-1α expression stimulated the expression of mRNA for all three key genes of gluconeogenesis: PEPCK, fructose 1,6-bisphosphatase, and glucose-6-phosphatase. Experiments performed at different doses of virus indicated that maximal induction of these genes occurred at approximately the same levels of PGC-1α protein that are present in fasted liver (51).

As might be predicted from the gene expression data, PGC-1α stimulated a 3-fold increase in the ability of hepatocytes to secrete glucose when provided with gluconeogenic precursors. Although this process was stimulated in control hepatocytes by 8-bromo-cAMP, no further augmentation was observed in the cells expressing PGC-1α via adenovirus, suggesting that a major effect of the hepatic cAMP was the elevation of PGC-1α.

Adenoviral-based vectors were also used to modify PGC-1α expression in the liver of rats *in vivo* (51). Rats were killed 5 d after tail-vein injection of virus expressing PGC-1α or green fluorescent protein. *Ad libitum*-fed rats that received the PGC-1α virus had an elevation of PGC-1α protein to approximately the levels seen in the fasted state of control animals. The PGC-1α recipient animals also had an elevation in PEPCK and glucose-6-phosphatase mRNA, as well as a significant rise in blood glucose and insulin. These data indicate that PGC-1α can regulate gluconeogenesis *in vivo* at physiological protein concentrations. cAMP response element binding protein (CREB), a transcription factor activated by glucagon and cAMP, directly activated the expression of PGC-1α through binding to its promoter. Indeed, overexpression of PGC-1α in CREB-deficient mice restored glucose homeostasis and rescued expression of gluconeogenic genes (52).

The PEPCK promoter has been used to study the mechanistic basis for PGC-1α's actions in gluconeogenesis. Utilizing transfections of the wild-type and mutant versions of the PEPCK promoter, coactivation of two nuclear receptors, hepatocyte nuclear factor-4α (HNF-4α) and the glucocorticoid receptor, appear to be important (51). The interaction with HNF-4α utilizes the activation function (AF)-2 domain of this receptor and the major LXXLL (amino acids 142–146) of PGC-1α. Interestingly, the interaction between LXXLL domain of coactivators and the AF-2 of most receptors is ligand dependent, but PGC-1α can coactivate HNF-4α without any addition of ligands.

In addition, one intriguing question is how insulin acts to suppress PGC-1α expression and whether this action of insulin is direct or via the counterregulatory hormones controlled by insulin: glucagons and the glucocorticoids. There is good evidence for the involvement of the protein kinase acute transforming retrovirus thymoma (AKT2) and Forkhead box protein 01 (FOXO1) transcription factor in the insulin suppression of gluconeogenesis (53, 54). Whether these function directly in PGC-1α gene expression or through other mechanisms remains to be determined.

III. Molecular Mechanisms of PGC- 1α Function

A. The PGC-1 gene gamily

PGC-1α is now part of a small family of transcriptional coactivators that includes the close homolog PGC-1β (32, 55) and PGC-1-related coactivator (Ref. 56 and Fig. 4). All these proteins share a high degree of homology in the N terminus and at the C terminus. The N terminus of all of these proteins contains a transcriptional activation domain and includes the

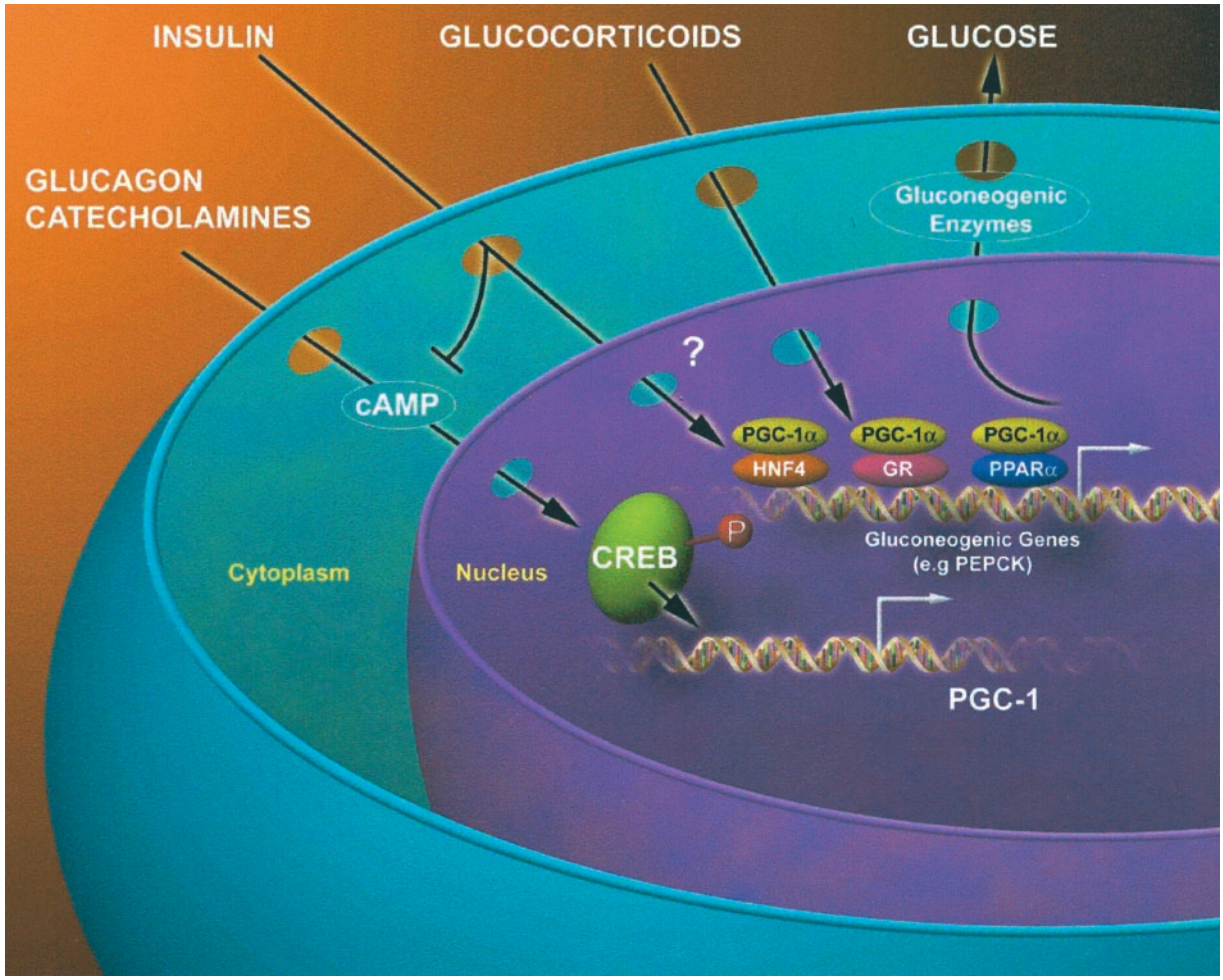

FIG. 3. Transcriptional regulation of hepatic gluconeogenesis by PGC-1α. Hepatic glucose production in the liver is tightly controlled by hormones. Glucagon and catecholamines stimulate the cAMP pathway and CREB, which activates PGC-1α gene expression. PGC-1α is then recruited to different transcription factors that bind to the promoter of gluconeogenic genes such as PEPCK. Glucocorticoids induce a specific interaction between PGC-1α and GR-activating PEPCK. Insulin represses cAMP activation on gluconeogenic genes, but how it blocks PGC-1α gene expression is unknown.

major nuclear hormone receptor-interacting motif (LXXLL). The C-terminal region contains an RNA-binding motif (RMM) and a serine-arginine-rich (RS) domain. Therefore, an unusual feature of the PGC-1 family is the presence of transcriptional activation domains and RNA processing motifs in the same molecule.

Computer-assisted searches reveal that domains at the N and C terminus of the PGC-1 family are conserved in proteins from Zebrafish and *Xenopus* to mammals, suggesting that these proteins may play important common roles in these species. It seems likely, based on what we know about PGC-1α, that these proteins in cold-blooded animals may be linked to mitochondrial function and/or regulation of other cellular processes related to energy metabolism.

It is important to note that it is not yet clear whether the biological functions described so far for PGC-1α can also be carried out by PGC-1β or PGC-1-related coactivator. Based on the cellular expression and regulations described (32), it is likely that they will overlap in some of the functions, but other new biological responses might be also expected.

B. Analysis of PGC-1α/transcription factor interactions

1. Interaction with different transcription factor families. Transcriptional coactivators are recruited to particular DNA sequences in gene promoters through direct interaction with transcription factors. PGC-1α was first identified as a protein of 798 amino acids that interacts with the nuclear receptor PPARγ, the master regulator of adipocyte differentiation (29). Structure-function analysis of PGC-1α revealed that the N-terminal 200 amino acids contain a potent transcriptional activation domain that is very rich in acidic amino acids (Fig. 4). Embedded in this region is an LXXLL sequence (amino acids 142–146), a motif known to be responsible for ligand-dependent interaction of other coactivators with nuclear hormone receptors (57). The LXXLL motif of PGC-1α belongs to the class III (58) and appears to interact with different amino acids within the AF-2 of the estrogen receptor (ER) compared with steroid receptor coactivator-1 (SRC-1), suggesting that there are structural differences between LXXLL motifs among the different coactivators. The LXXLL motif on PGC-1α is

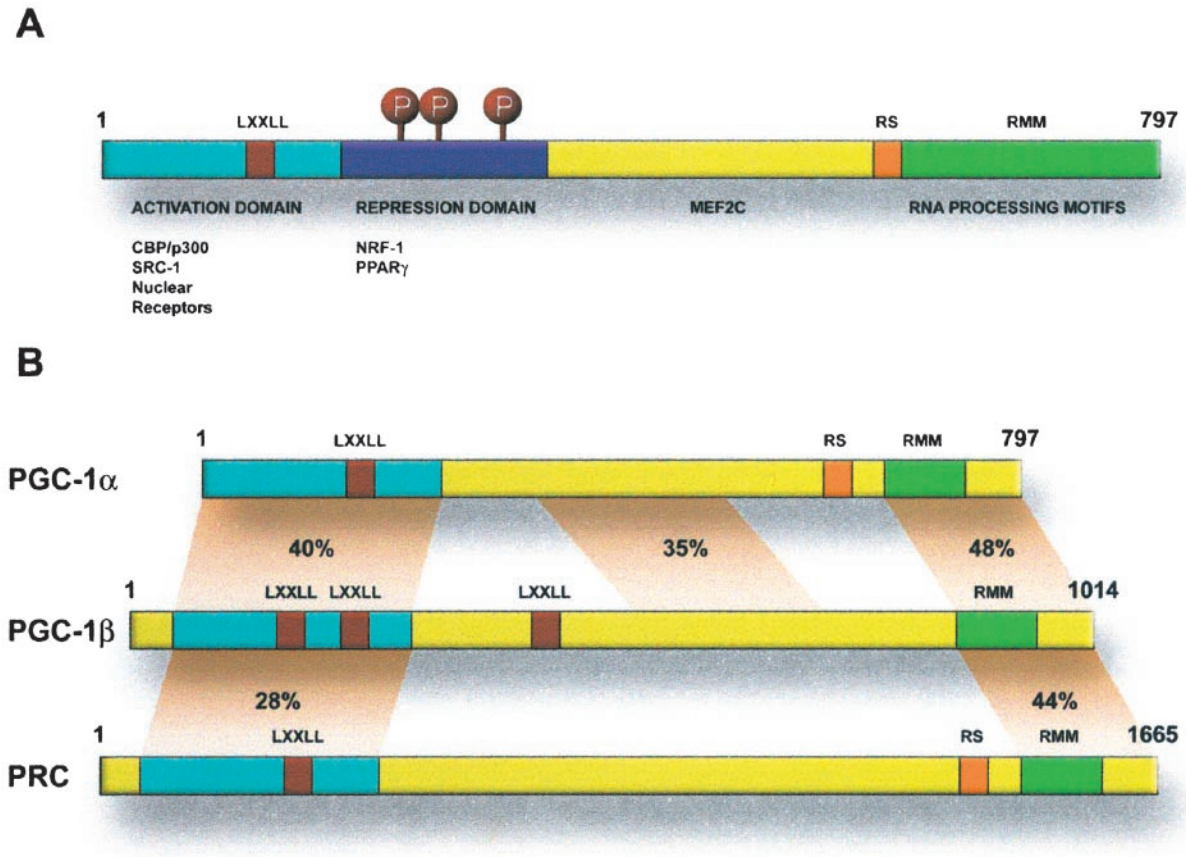

FIG. 4. Functional domains of the PGC-1 gene family. A, Architecture of PGC-1α protein. PGC-1α contains a potent activation domain at its N terminus that interacts with other transcriptional coactivators. The LXXLL motif is responsible for ligand-dependent interaction with certain hormone nuclear receptors. A central suppression domain contains several p38 MAPK phosphorylation sites. The C terminus contains RNA processing motifs such as an RS domain and an RMM. B, Protein sequence alignment of the PGC-1 gene family. The percentage homology between the different domains of PGC-1 proteins is indicated. Note that the more conserved domains are at the N terminus and C terminus.

absolutely required for the ligand-dependent interaction with ER (59), PPARα (48), RXRα (60), glucocorticoid receptor (GR; Ref. 61), and probably other nuclear hormone receptors. Interestingly, HNF4α also interacts with this motif of PGC-1α without addition of any ligand, suggesting that this nuclear hormone receptor is in an active conformation even without the addition of exogenous ligand (51). PGC-1α also uses different non-LXXLL domains to interact with certain other transcription factors: a domain between amino acids 200 and 400 interacts with PPARγ (29) and NRF-1 (37) and a region between amino acids 400 to 500 that interacts with MEF2-C (50). More detailed analyses of these interactions are required to better define these binding domains.

2. PCG-1 coactivates PPARγ in a promoter-specific manner. PGC-1α interacts with several nuclear hormone receptors present in the UCP-1 enhancer. The nature of these interactions can be ligand dependent, as in the case of RAR or TR, or ligand independent as with PPARγ (29). When the interaction between PGC-1α and a nuclear receptor is not ligand regulated, PGC-1α appears to interact with the central hinge region of the receptor; when this interaction is ligand dependent, interaction with PGC-1α appears to be with the C-terminal AF-2 region of the receptor.

We have shown that PGC-1α coactivates both PPARγ and the thyroid receptor in the UCP-1 enhancer. This activation was clearly synergistic after stimulation of cells with cAMP, a potent inducer of UCP-1 gene expression. If these mechanisms were very simple, one could expect that any coactivator would induce all the target genes of particular nuclear receptors without any promoter specificity. However, because not all promoters of endogenous genes with functional PPARγ binding sites are activated by PGC-1α, there is apparent promoter specificity even when this coactivator is working through the same nuclear receptor. A good example of this is the selectivity shown with the genes for UCP-1 and adipocyte protein 2 (aP2). Expression of PGC-1α in white fat cells turn on UCP-1 gene expression but failed to activate aP2 gene (29), the first gene actually identified as a target of PPARγ (62). This clearly illustrates that transcriptional coactivators are not only boosters of transcription but also play a key role in specification of the gene targeted by a transcription factor. Similar conclusions have been drawn by other investigators using chromatin immunoprecipitation analysis of the ER target genes, showing that in different promoters the ER binds to different coactivators (63). The molecular basis of this phenomena is currently unknown.

C. PGC-1α recruits other histone acetyl transferase (HAT)-containing coactivator proteins

Most coactivators potentiate transcriptional activity by having specific enzymatic functions that are required to remodel chromatin and initiate transcription (64–66). PGC-1α does not have significant amino acid sequence homology to other transcriptional coactivator families. In addition, PGC-1α does not contain any recognizable HAT domain and does not appear to have this intrinsic enzymatic activity. However, we have shown that the N-terminal activation domain of PGC-1α recruits proteins that contain HAT activity, such as SRC-1 and CREB binding protein (CBP/p300; Ref. 67). In addition, PGC-1α is also present in a complex containing RNA polymerase II. Whether PGC-1α is also part of the TR-associated proteins (TRAP)/vitamin-D receptor interacting proteins/activated recruited cofactor or the brahma-related gene 1/human brahma complexes is unknown.

The model of recruitment of transcriptional coactivators described above portrays the DNA binding transcription factor in a largely passive role, functioning mainly to localize the coactivator complexes to genes that are marked for activation. Conversely, the coactivator complex has been thought to be constitutively active, requiring only proper positioning in the genome to initiate transcription. We have investigated the mechanism of transcriptional activation and a potential role for transcription factor docking to PGC-1α. Interestingly, the binding of SRC-1 and CBP/p300 to the N terminus of PGC-1α is dependent upon docking of transcription factors such PPARγ and NRF-1 to the amino acids 200–400 region of PGC-1α (67). This suggests that PGC-1α is in a relatively quiescent state when not bound to a transcription factor; however, it becomes highly active when the transcription factor binds and induces a conformation change that recruits SRC-1 and CBP/p300 into the complex. Thus, transcription factor docking switches on the activity of this coactivator protein. Whether this phenomenon of transcriptional activation via a transcription factor docking event is a common feature of coactivators remains to be determined.

D. Interaction of PGC-1α with RNA processing complexes

A large body of evidence has suggested that pre-mRNA splicing is linked to transcription *in vivo* (68, 69). However, the molecular mechanisms that couple transcription and mRNA processing have not been elucidated. The fact that PGC-1α contains domains involved in splicing and other domains involved in binding to transcriptional factors and other coactivators makes PGC-1α an excellent candidate to study the integration of these two processes.

The C terminus of PGC-1α contains two domains involved in RNA processing: a RS domain at amino acids 565–631 and an RMM domain at amino acids 677–709 (29). As mentioned above, proteins containing paired RNA-binding motifs and RS domains have been shown to interact with the C-terminal domain of RNA polymerase II, indicating a putative link between transcription and RNA processing (70). In fact, we have shown that PGC-1α is in a complex with the phosphorylated form of RNA polymerase II and other factors involved in elongation such as cyclin-dependent kinase 9 and cyclin T. The C-terminal domain of PGC-1α that contains the RS and RMM was responsible for maintaining these interactions (71).

PGC-1α contains two putative nuclear localization signals and localizes to the cell nucleus (amino acids 326–333, 627–633, and 651–667). The localization in the nucleus is not homogenous but is more concentrated in nuclear speckles, typical of splicing factors. This localization to speckles is lost in mutants that lack the RS and RMM domains. Indeed, PGC-1α is in a complex with splicing factors such as splicing component, 35 kDa (SC35); serine/arginine protein, 75 kDa (SRp75); serine/arginine protein, 55 kDa (SRp55); and serine/arginine protein, 40 kDa (SRp40), suggesting that this coactivator can participate in the RNA splicing process. In addition, we have also shown that PGC-1α modulates both expression and processing of a target fibronectin mini-gene (71). Interestingly, PGC-1α does not participate in RNA processing unless it is involved in the transcription of that particular gene. It would be interesting to identify specific RNA targets to which PGC-1α binds and the mechanisms by which processing and/or stabilization functions are controlled.

E. Phosphorylation of PGC-1α by p38 MAPK

It is well established that transcription factors are targets of multiple signal transduction pathways, but there is also emerging evidence that coactivators can also be targets of these signaling pathways in response to growth factors, hormones, and ligands. For example, it has been shown that CBP transcriptional activity is enhanced in response to calcium signaling through calmodulin kinase IV (72). In addition, SRC-1 has been shown to be a target of cAMP signaling through protein kinase A (PKA; Ref. 73). Therefore, regulation of transcriptional coactivator function provides a target to integrate different responses to specific signals across multiple transcription factors.

As discussed in more detail in *Section II.B*, the role of cytokines in energy expenditure caused us to examine the effects of protein kinases downstream of cytokines on PGC-1α structure and function. We have found that p38 stress-activated MAPK phosphorylates PGC-1α in three residues (T262, S265, and T298; Ref. 45). Indeed, it was shown that phosphorylation of PGC-1α on those residues leads to increased stability and half-life. It is interesting that these phosphorylations occur in a region previously shown to play an important regulatory role in PGC-1α binding to transcription factors. Earlier work (67) has illustrated that this region (amino acids 200–403) mediates a repressive effect in transcription and also serves to dock several transcription factors, including many nuclear receptors and NRF-1. The docking of these transcription factors causes a conformational change that accelerates the binding of other transcriptional effector proteins into this complex, including CBP/p300 and SRC-1 (67). Whether the p38 MAPK-mediated phosphorylations affect transcription factor docking or the recruitment of other coactivator proteins to a PGC-1α complex remains to be determined. Furthermore, how these phospho-amino acids change protein stability is currently under investigation.

Kralli's group (74) has also shown that activation of p38 MAPK leads to an increase in transcriptional activity of PGC-

1α. They hypothesize the existence of a putative repressor that binds to the PGC-1α LXXLL motif and that the interaction is released upon activation of p38 MAPK. Interestingly, it has been shown that PPARα/PGC-1α transcriptional activity can be activated through the p38 MAPK pathway, having possible physiological roles in heart stress conditions (75). However, the precise mechanism by which p38 MAPK modification of PGC-1α alters the amount and activity of this coactivator remains to be determined and could have important therapeutic implications.

F. Model of PGC-1α functions in gene expression

How does PGC-1α activate specific gene targets? The N-terminal transcriptional activation domain of PGC-1α and additional regions bind to DNA-binding proteins and recruit proteins with HAT activity, strongly indicating PGC-1α's involvement in transcription initiation on promoters of particular genes. Two conserved protein domains in the C terminus of PGC-1α that have homology to proteins involved in RNA processing, as well as the interaction of PGC-1α with splicing and elongation factors, would also indicate its participation in posttranscriptional initiation processes (Fig. 5). Taken together, three possible models could explain how PGC-1α affects the activation of particular target genes. In some cases, PGC-1α could act as a classical transcriptional activator, binding to specific transcription factors and increasing rates of transcription initiation. In this model, PGC-1α would remain bound to the promoter through the transcription factor. One interesting possibility here is that PGC-1α could also provide some degree of promoter specificity to the transcription factor. In a second model, PGC-1α could affect certain genes not by affecting transcriptional initiation but by targeting RNA processing functions such as elongation, mRNA capping, alternative mRNA splicing, or even mRNA stability. A third model would combine these two models: PGC-1α is a dynamic player involved in several different aspects of gene expression. PGC-1α it would first be loaded at the promoter region through transcription factor docking and recruitment of HAT complexes. Once transcriptional initiation has started, PGC-1α would be involved in the elongation process through interaction with elongation factors and phosphorylated RNA polymerase II, presumably affecting the rates of RNA elongation and/or splicing.

One interesting question highlighted here is why would there be this tight linkage of transcription and RNA processing? One possible explanation is the requirement for chromatin remodeling enzymes to become processive and open chromatin structure over the entire length of a given gene. It is conceivable that linking the coactivators, active in the initiation of transcription, to RNA polymerase and the RNA processing machinery functions to allow proteins such as CBP/p300 to roll down the DNA and acetylate histones over all necessary coding segments. This "HATs on wheels" model has not yet been tested.

IV. Concluding Remarks and Future Perspectives

At first glance, it appears that PGC-1α is involved in a somewhat bewildering array of disparate biological responses. However, a common theme is that PGC-1α promotes patterns of gene expression that favor oxidative metabolism in various forms. Clearly, brown fat differentiation involves the execution of a program that emphasizes mitochondrial-based fuel oxidation to generate heat. Similarly, the ability of PGC-1α to promote mitochondrial biogenesis and respiration in skeletal muscle indicates an important choice to utilize oxidative, as opposed to glycolytic, metabolism. Interestingly, skeletal muscle has different fiber types that differ greatly in these parameters. Indeed, slow-twitch muscle is composed primarily of type I fibers that are much richer in mitochondria and more dependent on oxidative metabolism. In contrast, fast-twitch muscle contains more type 2b fibers that rely more on glycolitic metabolism. Preliminary data in our laboratory suggest that PGC-1α is ex-

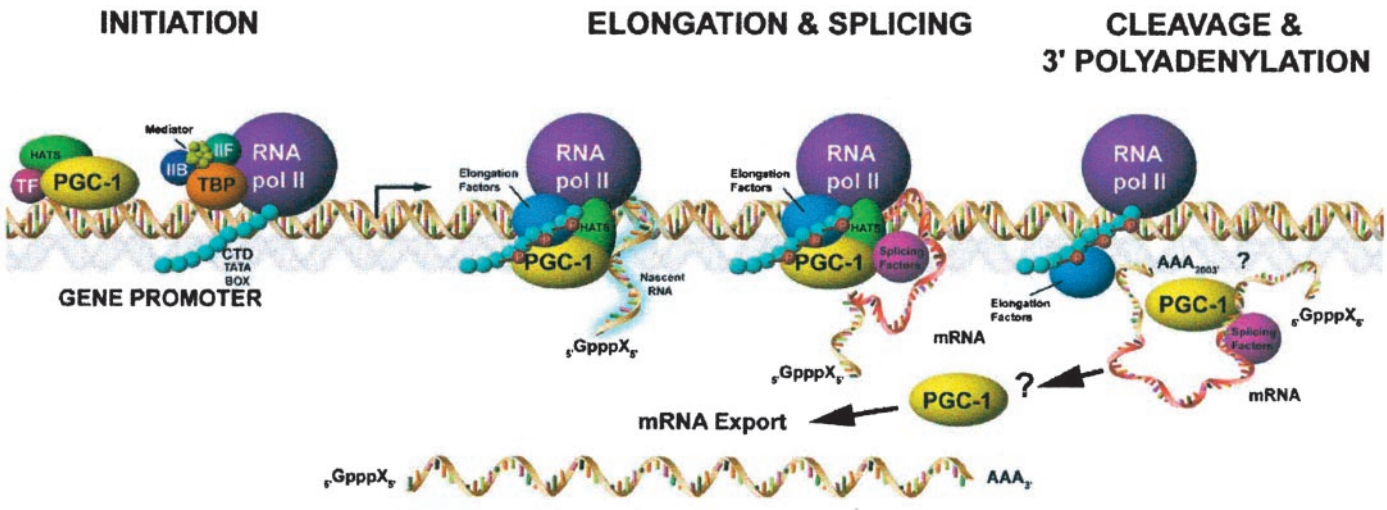

FIG. 5. Model of PGC-1α-mediated gene activation. PGC-1α is a dynamic molecule that is involved in different steps of gene expression. It is loaded at gene promoters through direct interaction with transcription factors. Once transcription has initiated, PGC-1α interacts with elongation and splicing factors participating in these processes. It is possible that PGC-1 carries the HAT proteins through the elongation process. Finally, it could also participate in polyadenylation, cleavage, and mRNA export through direct binding to the mRNA.

pressed at much higher levels in slow-twitch muscle, indicating a possible role in some aspects of fiber-type decisions.

The role of PGC-1α in hepatic glucose output seems quite distinct in that gluconeogenesis is not an oxidative response. However, it must be recalled that gluconeogenesis is just one part of the fasted response in liver, a state that certainly does involve a greatly increased level of β-oxidation of fatty acids in mitochondria. A role for PGC-1α in this program of β-oxidation has previously been shown in fat and cardiac cells, and it is highly likely to be true in liver as well. Hence, although it is certainly not a differentiation response *per se*, it is very likely that PGC-1α plays a role in the physiological switch in liver between the fed and fasted states.

Because of the role of PGC-1α in many important metabolic processes, it is worth asking whether and how the activities of PGC-1α might be modulated for therapeutic purposes. PGC-1α's function in the BAT thermogenic program, regulation of Glut-4 and mitochondrial oxidation in muscle, and the apparently dominant role of PGC-1α in hepatic gluconeogenesis all suggest that this coactivator could be a target for antiobesity or diabetes drugs. Of course, this wide range of physiological actions also points out a problem: activation or inhibition is going to have to be tissue selective or tissue specific to be useful. For example, it is essentially established that drugs that inhibit gluconeogenesis in liver can have a profound antidiabetic action in humans. Hence, an inhibitor of PGC-1α could theoretically ameliorate this disorder. Unfortunately, mitochondrial biogenesis and oxidation, probably influenced and controlled by PGC-1α, is critical for the health of heart, skeletal muscle, and many other tissues. This suggests that the usefulness of PGC-1α as a therapeutic target will all come down to an ability to achieve biological specificity.

However, because PGC-1α is a coactivator and is dependent on the docking on particular transcription factors to execute different biological programs, inhibition of different docking events should affect some, but not all, biological responses. The ability of PGC-1α to stimulate gluconeogenesis seems to depend on PGC-1α's physical interaction with HNF-4α and the GR. The activation of mitochondrial biogenesis in skeletal muscle is dependent upon the docking on NRF-1. Thus, targeting specific docking events between PGC-1α and particular transcription factors might offer new theoretical possibilities to control metabolism; where this involves nuclear receptors, as in gluconeogenesis, opportunities might be greater as agonists or antagonists of these receptors with specificity for PGC-1 might be developed.

Acknowledgments

We thank members of the Spiegelman laboratory for helpful discussions, Brad Lowell and Cliff Yoon for reading the manuscript, and Eric Smith for the graphical support for the figures presented in this review.

Address all correspondence and requests for reprints to: Dr. Bruce M. Spiegelman, Dana-Farber Cancer Institute, One Jimmy Fund Way, Smith Building, Room 958, Boston, Massachusetts 02115. E-mail: bruce_spiegelman@dfci.harvard.edu

P.P. is supported by a Lee Investigation Career Award. B.M.S. is supported by NIH Grants DK54477 and R37DK31405.

References

1. **Lowell BB, Spiegelman BM** 2000 Towards a molecular understanding of adaptive thermogenesis. Nature 404:652–660
2. **Cannon B, Houstek J, Nedergaard J** 1998 Brown adipose tissue. More than an effector of thermogenesis? Ann NY Acad Sci 856: 171–187
3. **Rothwell NJ, Stock MJ** 1979 A role for brown adipose tissue in diet-induced thermogenesis. Nature 281:31–35
4. **Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP** 1997 Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387:90–94
5. **Cannon B, Jacobsson A, Rehnmark S, Nedergaard J** 1996 Signal transduction in brown adipose tissue recruitment: noradrenaline and beyond. Int J Obes Relat Metab Disord 20(Suppl 3):S36–S42
6. **Ricquier D** 1989 Molecular biology of brown adipose tissue. Proc Nutr Soc 48:183–187
7. **Silva JE** 1995 Thyroid hormone control of thermogenesis and energy balance. Thyroid 5:481–492
8. **Reitman ML, He Y, Gong DW** 1999 Thyroid hormone and other regulators of uncoupling proteins. Int J Obes Relat Metab Disord 23(Suppl 6):S56–S59
9. **Zurlo F, Larson K, Bogardus C, Ravussin E** 1990 Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest 86:1423–1427
10. **Zurlo F, Nemeth PM, Choksi RM, Sesodia S, Ravussin E** 1994 Whole-body energy metabolism and skeletal muscle biochemical characteristics. Metabolism 43:481–486
11. **Simonsen L, Bulow J, Madsen J, Christensen NJ** 1992 Thermogenic response to epinephrine in the forearm and abdominal subcutaneous adipose tissue. Am J Physiol 263:E850–E855
12. **Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier, D, Warden, CH** 1997 Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 15:269–272
13. **Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, Woolf EA, Tartaglia LA** 1997 Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. Diabetes 46:900–906
14. **Boss O, Samec S, Paoloni-Giacobino A, Rosier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP** 1997 Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408:39–42
15. **Gong DW, He Y, Karas M, Reitman M** 1997 Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. J Biol Chem 272:24129–24132
16. **Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB** 1997 UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. Biochem Biophys Res Commun 235:79–82
17. **Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, Lowell BB** 2000 Energy metabolism in uncoupling protein 3 gene knockout mice. J Biol Chem 275:16258–16266
18. **Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D** 2000 Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 26:435–439
19. **Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB** 2001 Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. Cell 105:745–755
20. **Cassard-Doulcier AM, Gelly C, Fox N, Schrementi J, Raimbault S, Klaus S, Forest C, Bouillaud F, Ricquier D** 1993 Tissue-specific and beta-adrenergic regulation of the mitochondrial uncoupling protein gene: control by *cis*-acting elements in the 5′-flanking region. Mol Endocrinol 7:497–506
21. **Kozak UC, Kopecky J, Teisinger J, Enerback S, Boyer B, Kozak LP** 1994 An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. Mol Cell Biol 14:59–67

22. **Cassard-Doulcier AM, Larose M, Matamala JC, Champigny O, Bouillaud F, Ricquier D** 1994 *In vitro* interactions between nuclear proteins and uncoupling protein gene promoter reveal several putative transactivating factors including Ets1, retinoid X receptor, thyroid hormone receptor, and a CACCC box-binding protein. J Biol Chem 269:24335–24342
23. **Barbera MJ, Schluter A, Pedraza N, Iglesias R, Villarroya F, Giralt M** 2001 Peroxisome proliferator-activated receptor α activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. J Biol Chem 276:1486–1493
24. **Sears IB, MacGinnitie MA, Kovacs LG, Graves RA** 1996 Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor γ. Mol Cell Biol 16:3410–3419
25. **Foellmi-Adams LA, Wyse BM, Herron D, Nedergaard J, Kletzien RF** 1996 Induction of uncoupling protein in brown adipose tissue. Synergy between norepinephrine and pioglitazone, an insulin-sensitizing agent. Biochem Pharmacol 52:693–701
26. **Tai TA, Jennermann C, Brown KK, Oliver BB, MacGinnitie MA, Wilkison WO, Brown HR, Lehmann JM, Kliewer SA, Morris DC, Graves RA** 1996 Activation of the nuclear receptor peroxisome proliferator-activated receptor γ promotes brown adipocyte differentiation. J Biol Chem 271:29909–29914
27. **Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM** 1999 PPAR γ is required for placental, cardiac, and adipose tissue development. Mol Cell 4:585–595
28. **Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM** 1999 PPAR γ is required for the differentiation of adipose tissue *in vivo* and *in vitro*. Mol Cell 4: 611–617
29. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829–839
30. **Boss O, Bachman E, Vidal-Puig A, Zhang CY, Peroni O, Lowell BB** 1999 Role of the β(3)-adrenergic receptor and/or a putative β(4)-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-γ coactivator-1. Biochem Biophys Res Commun 261:870–876
31. **Gomez-Ambrosi J, Fruhbeck G, Martinez JA** 2001 Rapid *in vivo* PGC-1 mRNA upregulation in brown adipose tissue of Wistar rats by a β(3)-adrenergic agonist and lack of effect of leptin. Mol Cell Endocrinol 176:85–90
32. **Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM** 2002 Peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 277:1645–1648
33. **Kozak LP** 2000 Genetic studies of brown adipocyte induction. J Nutr 130:3132S–3133S
34. **Nelson BD** 1990 Thyroid hormone regulation of mitochondrial function. Comments on the mechanism of signal transduction. Biochim Biophys Acta 1018:275–277
35. **Evans MJ, Scarpulla RC** 1990 NRF-1: a trans-activator of nuclear-encoded respiratory genes in animal cells. Genes Dev 4:1023–1034
36. **Virbasius JV, Virbasius CA, Scarpulla RC** 1993 Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. Genes Dev 7:380–392
37. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell BB, Scarpulla RC, Spiegelman BM** 1999 Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115–124
38. **Tsukiyama-Kohara K, Poulin F, Kohara M, DeMaria CT, Cheng A, Wu Z, Gingras AC, Katsume A, Elchebly M, Spiegelman BM, Harper ME, Tremblay ML, Sonenberg N** 2001 Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat Med 7:1128–1132
39. **Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, Enerback S** 2001 FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 106:563–573
40. **Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A** 1987 Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330: 662–664
41. **Tracey KJ, Cerami A** 1993 Tumor necrosis factor: an updated review of its biology. Crit Care Med 21:S415–422
42. **Mercurio F, Manning AM** 1999 Multiple signals converging on NF-κB. Curr Opin Cell Biol 11:226–232
43. **Davis RJ** 2000 Signal transduction by the JNK group of MAP kinases. Cell 103:239–252
44. **Chang L, Karin M** 2001 Mammalian MAP kinase signalling cascades. Nature 410:37–40
45. **Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM** 2001 Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARγ coactivator-1. Mol Cell 8:971–982
46. **Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP** 2000 Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 106: 847–856
47. **Sack MN, Disch DL, Rockman HA, Kelly DP** 1997 A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. Proc Natl Acad Sci USA 94:6438–6443
48. **Vega RB, Huss JM, Kelly DP** 2000 The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20:1868–1876
49. **Randle PJ, Priestman DA, Mistry SC, Halsall A** 1994 Glucose fatty acid interactions and the regulation of glucose disposal. J Cell Biochem 55(Suppl):1–11
50. **Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmant G, Lehman JJ, Kelly DP and Spiegelman BM** 2001 Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proc Natl Acad Sci USA 98:3820–3825
51. **Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM** 2001 Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131–138
52. **Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman BM, Montminy M** 2001 CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413:179–183
53. **Nakae J, Kitamura T, Silver DL, Accili D** 2001 The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 108:1359–1367
54. **Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ** 2001 Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). Science 292:1728–1731
55. **Kressler D, Schreiber SN, Knutti D, Kralli A** 2002 The PGC-1 -related protein PERC is a selective coactivator of estrogen receptor α. J Biol Chem 277: 13918–13925
56. **Andersson U, Scarpulla RC** 2001 Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. Mol Cell Biol 21: 3738–3749
57. **Heery DM, Kalkhoven E, Hoare S, Parker MG** 1997 A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733–736
58. **Chang C, Norris JD, Gron H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP** 1999 Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors alpha and beta. Mol Cell Biol 19:8226–8239
59. **Tcherepanova I, Puigserver P, Norris JD, Spiegelman BM, McDonnell DP** 2000 Modulation of estrogen receptor-alpha transcriptional activity by the coactivator PGC-1. J Biol Chem 275: 16302–16308
60. **Delerive P, Wu Y, Burris TP, Chin WW, Suen CS** 2001 PGC-1 functions as a transcriptional coactivator for the retinoid X receptors. J Biol Chem 277: 3913–3917
61. **Knutti D, Kaul A, Kralli A** 2000 A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. Mol Cell Biol 20:2411–2422

62. **Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM** 1994 mPPAR γ 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 8:1224–1234
63. **Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M** 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852
64. **Struhl K** 1998 Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12:599–606
65. **Glass CK, Rosenfeld MG** 2000 The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141
66. **Naar AM, Lemon BD, Tjian R** 2001 Transcriptional coactivator complexes. Annu Rev Biochem 70:475–501
67. **Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman BM** 1999 Activation of PPARγ coactivator-1 through transcription factor docking. Science 286:1368–1371
68. **Neugebauer KM, Roth MB** 1997 Transcription units as RNA processing units. Genes Dev 11:3279–3285
69. **Corden JL, Patturajan M** 1997 A CTD function linking transcription to splicing. Trends Biochem Sci 22:413–416
70. **Yuryev A, Patturajan M, Litingtung Y, Joshi RV, Gentile C, Gebara M, Corden JL** 1996 The C-terminal domain of the largest subunit of RNA polymerase II interacts with a novel set of serine/arginine-rich proteins. Proc Natl Acad Sci USA 93:6975–6980
71. **Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M, Spiegelman BM** 2000 Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. Mol Cell 6:307–316
72. **Chawla S, Hardingham GE, Quinn DR, Bading H** 1998 CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science 281:1505–1509
73. **Rowan BG, Garrison N, Weigel NL, O'Malley BW** 2000 8-Bromocyclic AMP induces phosphorylation of two sites in SRC-1 that facilitate ligand-independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CREB binding protein. Mol Cell Biol 20:8720–8730
74. **Knutti D, Kressler D, Kralli A** 2001 Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. Proc Natl Acad Sci USA 98:9713–9718
75. **Barger PM, Browning AC, Garner AN, Kelly DP** 2001 p38 Mitogen-activated protein kinase activates peroxisome proliferator-activated receptor α. A potential role in the cardia metabolic stress response. J Biol Chem 276:44495–44501
76. **Ge H, Guermah M, Yuan CX, Ito M, Walberg AE, Spiegelman BM, Roeder RG** 2002 The TRAP220 subunit of the TRAP/mediator is required for PPARγ2-stimulated adipogenesis. Nature 417: 563–567