0163-769X/08/$20.00/0
Printed in U.S.A.

Endocrine Reviews 29(6):677–696

doi: 10.1210/er.2008-0017

Transcriptional Control of Energy Homeostasis by the Estrogen-Related Receptors

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Transcriptional control of cellular energy metabolic pathways is achieved by the coordinated action of numerous transcription factors and associated coregulators. Several members of the nuclear receptor superfamily have been shown to play important roles in this process because they can translate hormonal, nutrient, and metabolite signals into specific gene expression networks to satisfy energy demands in response to distinct physiological cues. Estrogen-related receptor (ERR) α, ERRβ, and ERRγ are nuclear receptors that have yet to be associated with a natural ligand and are thus considered as orphan receptors. However, the transcriptional activity of the ERRs is exquisitely sensitive to the presence of coregulatory proteins known to be essential for the control of energy homeostasis, and for all intents and purposes, these coregulators function as protein ligands for the ERRs. In particular, functional genomics and biochemical studies have shown that ERRα and ERRγ operate as the primary conduits for the activity of members of the family of PGC-1 coactivators. As transcription factors, the ERRs control vast gene networks involved in all aspects of energy homeostasis, including fat and glucose metabolism as well as mitochondrial biogenesis and function. Phenotypic analyses of knockout mouse models have shown that all three ERRs are indispensable for proper development and/or survival of the organism when subjected to a variety of physiological challenges. The focus of this review is on the recent and rapid advances in understanding the functions of the ERRs in regulating bioenergetic pathways, with an emphasis on their roles in the specification of energetic properties required for cell- and tissue-specific functions. (*Endocrine Reviews* 29: 677–696, 2008)

I. Introduction

ENERGY HOMEOSTASIS IS the balanced regulation of fuel intake, storage, and expenditure by an organism. Although a constant supply of energy is required to maintain the basal metabolic rate of the whole organism, energy is also differentially generated and used by individual cell types and organs in response to endogenous or exogenous signals specified by various developmental and physiological needs and stimuli. Regulated control of energy production and utilization thus allows the organism to function effectively in a broad range of environmental conditions. On the other hand, disturbance of energy homeostasis over a long period of time can contribute to the etiology of many diseases such as diabetes, obesity, and heart failure. Imbalance in metabolic homeostasis may originate from nutritional deprivation or

First Published Online July 29, 2008

Abbreviations: AF, Activation function; apo, apolipoprotein; BAT, brown adipose tissue; ChIP, chromatin immunoprecipitation; CoA, coenzyme A; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; DBD, DNA-binding domain; ER, estrogen receptor; ERR, estrogen-related receptor; ERRE, ERR response element; FAO, fatty acid oxidation; FOXO1, forkhead box O1; FXR, farnesoid X receptor; GABP, GA-binding protein; HIF, hypoxia-inducible factor; HNF4α, hepatocyte nuclear factor 4α; IFN-γ, interferon γ; LBD, ligand binding domain; LXR, liver X receptor; MCAD, medium-chain acyl-coenzyme A dehydrogenase; MEFs, mouse embryonic fibroblasts; NRF-2, nuclear respiratory factor-2; NRRE, nuclear receptor response element; NTD, amino terminal domain; OXPHOS, oxidative phosphorylation; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PEPCK, phosphoenolpyruvate carboxykinase; PGC, PPARγ coactivator; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RIP140, receptor interacting protein 140; ROS, reactive oxygen species; RXR, retinoid X receptor; SHP, small heterodimer partner; siRNA, small inhibitory RNA; SRC, steroid receptor coactivator; TCA, tricarboxylic acid cycle; TR, thyroid hormone receptor; TRP53 (or p53), transformation-related protein 53; UCP, uncoupling protein; WAT, white adipose tissue.

***Endocrine Reviews* is published by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

overabundance, genetic variations and disorders, as well as from gradual loss of efficiency in control systems during the aging process. Consequently, improving our knowledge of how metabolic homeostasis can be maintained through the life of the organism and defining, at the molecular level, control mechanisms that can be manipulated to prevent and treat metabolic-related diseases are of indisputable medical significance.

Complex biochemical pathways have evolved to adequately respond to the constantly changing metabolic needs of individual cells, specific organs, or the whole organism. Homeostatic regulation is mostly under hormonal control and is achieved through allosteric control and posttranslational modifications of key metabolic enzymes for immediate responses. However, long-term adaptation requires transcriptional regulation of metabolic genes (1). Nuclear receptors, a family of 48 transcription factors in humans that respond to small lipophilic hormones, vitamins, and metabolites, possess the ability to directly regulate gene expression (2). For example, the roles of glucocorticoids and thyroid hormones and their cognate nuclear receptors in the regulation of glucose metabolism and basal metabolic rate, respectively, have been studied for decades (3). More recently, lipid metabolites such as fatty acids, hydroxy-cholesterols, and bile acids have been identified as ligands for the peroxisome proliferator-activated receptor (PPAR) isoforms (PPARα, β, and γ, or NR1C1, NR1C2, and NR1C3), the liver X receptor α and β (LXRα and β, or NR1H3 and NR1H2), and the farnesoid X receptor (FXR, or NR1H4), respectively (4, 5). These nuclear receptors have been implicated in the regulation of gene networks involved in metabolic processes such as fatty acid oxidation (FAO), lipogenesis, thermogenesis, cholesterol homeostasis, and bile acid synthesis (1). LXR has also been proposed as an intracellular sensor for glucose (6). In contrast, several members of the superfamily of nuclear receptors still have no known natural ligands and remain classified as orphan receptors. Despite the absence of natural ligands that could link these receptors to specific functions and metabolic pathways, several orphan receptors have been directly implicated in the control of metabolism (1, 7, 8). For example, the chicken ovalbumin upstream promoter-transcription factor 1 (COUP-TF 1 or NR2F1) influences the expression of *Pck1*, the gene encoding phosphoenolpyruvate carboxykinase (PEPCK) (9), hepatocyte nuclear factor 4α (HNF4α or NR2A1) affects the expression of several genes involved in glucose, fatty acid, and cholesterol metabolism (10), and members of the NR4A subfamily (NGFI-B or NR4A1, Nurr1 or NR4A2, and NOR-1 or NR4A3) regulate energy metabolism in response to sympathetic nervous system signaling (8). Thus, orphan nuclear receptors may occupy a position equal to their liganded counterparts in the regulation of metabolic processes.

This review will focus on a specific class of orphan nuclear receptors known as the estrogen-related receptors (ERRs). A large body of recent work has established that the ERRs play a surprisingly central role in regulating energy metabolism. First, the ERRs are expressed in tissues associated with lipid metabolism and high energy demands; second, their transcriptional activity is highly dependent on the presence of nuclear receptor coregulators implicated in the control of complex metabolic programs; third, genetic studies in mice revealed that their presence is essential for the generation of energy and related tissue-specific functions; and fourth, functional genomics/proteomics studies have associated ERRs with the control of vast metabolic gene networks, in particular with those involved in mitochondrial biogenesis and function. Herein, a brief overview of the key features of members of the ERR subfamily is first provided. Then, the review describes how identification of target genes and phenotypic analyses of ERR-null mice linked the ERRs to the control of energy metabolism. Finally, the potential relevance of regulating ERRs activity in human diseases is discussed.

II. The Estrogen-Related Receptors

The ERR subfamily represents the archetype of orphan nuclear receptors. Several comprehensive reviews have described the structural and functional features of the ERRs as well as their potential interactions with estrogen signaling pathways (5, 11–16). The next sections will thus only highlight the attributes of ERRs necessary to understand how these receptors, acting as transcription factors, can regulate energy metabolism.

A. Structural and functional features of the ERRs

The first two members of the family, ERRα and ERRβ (NR3B1 and NR3B2), were identified from kidney and heart in a screen designed to clone steroid hormone receptors closely related to the estrogen receptor (ER) α (NR3A1) (17). However, it soon became apparent that the two new members of the superfamily of nuclear receptors did not possess the ability to bind a broad range of known steroid hormones or their derivatives and were thus recognized as the first orphan nuclear receptors. The third member of the ERR subfamily, ERRγ (NR3C3), was identified a decade later as the last member of the superfamily (18–20). ERRα and ERRγ can then be considered as the two bookends of the superfamily of nuclear receptors. In addition to the main three isoforms, several splice variants of ERRβ and ERRγ have been identified in humans (12). It is currently unknown whether these spliced variants play specific roles in tissues in which they are expressed. All ERR isoforms share the characteristic structural features of nuclear receptors, including a nonconserved amino terminal domain (NTD), a central DNA-binding domain (DBD) composed of two zinc finger motifs, and a functional ligand-binding domain (LBD) embedding docking sites for nuclear receptor coregulators (Fig. 1A).

1. The amino terminal domain. The NTD of nuclear receptors is a nonconserved and unstructured region that can contain an activation function (AF)-1 and is often subjected to posttranslational modifications. In contrast to other members of the superfamily, the three ERR isoforms share considerable amino acid identity in their NTDs, suggesting an important role for the domain in the regulation of ERR transcriptional activities. Functional characterization of the ERRs has yet to clearly identify a functional AF-1 within the NTD, but its posttranslational modification can affect ERR transcriptional

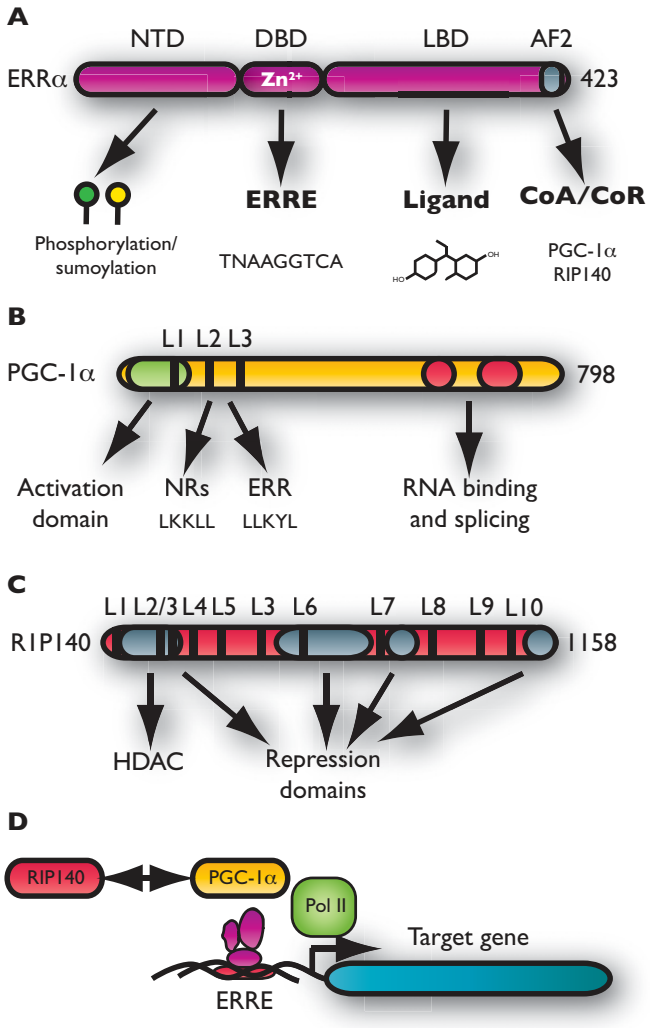

FIG. 1. ERRα and its main partners in gene regulation. A, Schematic representation of ERRα depicting its functional domains. The NTD is a site for posttranslational modifications, the DBD contains two zinc finger domains that recognize the consensus ERRE, and the LBD possesses a functional ligand binding pocket and an AF-2 (*blue*) that interacts with the coactivator PGC-1α and corepressor RIP140. B, Linear representation of PGC-1α with its activation domain (*green*) and its RNA binding and splicing domains (*red*). The location of the three LxxLL nuclear receptor recognition motifs is indicated by L1, L2, and L3. C, RIP140 is shown with its four repression domains (*gray*) and 10 putative LxxLL nuclear receptor recognition motifs. HDAC, Histone deacetylase. D, Model of ERRα-mediated gene regulation. The receptor recognizes target genes via binding to an ERRE located in a regulatory region located in the promoter or at a distant site. ERRα interacts either with PGC-1α or RIP140 to activate or repress gene transcription. Additional complexes (not shown) are presumably required to actively repress or enhance transcription of the target gene.

activities. Serine residues 19 and 22 in ERRα are phosphorylated, and mutation of serine 19 to a nonphosphorylatable alanine residue increases the transcriptional activity of the receptor in response to the presence of coactivators (21, 22). Phosphorylation of serine 19 is required for sumoylation of lysine 14, and mutation of serine 19 to an arginine residue also leads to an increase in ERRα activity. Indeed, the NTD of both ERRα and ERRγ contains a functional phospho-sumoyl switch motif (21, 23). This motif, ψKxEPxSP, is embedded within a larger synergy control motif that modulates higher order interactions among transcription factors (24). Accordingly, the increased transcriptional activity of the sumoylation-deficient ERRα mutants is more pronounced in the presence of multiple ERR binding sites (21). Although the precise signals modulating ERR phosphorylation and sumoylation remain to be identified, the observation that PPARγ is the only other member of the nuclear receptor superfamily to possess a phospho-sumoyl switch motif within its NTD suggests a putative link between phosphorylation-dependent sumoylation and transcriptional control of energy metabolism by nuclear receptors.

2. The DNA binding domain. Nuclear receptors recognize short DNA sequences, referred to as hormone response elements, located in both distal and proximal regulatory regions of target genes. The receptors bind DNA as monomer, homodimer, or heterodimer entities. Each monomeric unit recognizes a short six base pairs core motif, and binding specificity is imparted by both the primary sequence of the core motifs and the spacing between the two motifs. Monomeric binding by nuclear receptors requires the recognition of three to six additional nucleotides adjacent to the core half-site. Unbiased binding site sequence identification showed that ERRα recognizes the consensus sequence TCAAGGTCA, a single core motif preceded by three nucleotides (25). Bioinformatics analysis of a large number of ERR target promoters identified through genome-wide chromatin immunoprecipitation (ChIP)-on chip assays recently confirmed the presence of this motif at most ERR binding regions (26). However, the ERRs have the ability to bind to this sequence, referred to as the ERR response element (ERRE), as a monomer or a homodimer or as heterodimeric complexes composed of two distinct ERR isoforms (25–31). The exact molecular mechanism by which ERR dimers can bind to a single core half-site instead of two has yet to be elucidated. It has also been shown that the ERRs can bind *in vitro* to the same hormone response element as the ER (32–34). It is currently unknown whether recognition of the estrogen response element by the ERRs has physiological significance in regard to metabolic control or other biological functions.

3. An orphan receptor with a functional LBD. The LBD of the ERRs contains a well-conserved AF-2 helix motif. The conformation of the AF-2 helix, either in the active or inactive state, is usually dictated by the presence of the natural ligand (35). However, elucidation of the crystal structures of ERRα and ERRγ LBDs showed that in the absence of a ligand, the AF-2 helix of both receptors is positioned in the active configuration (36–39). Thus, the ERRs have the capacity to interact with coactivators in a ligand-independent manner. This observation suggests that the relative concentration of a coactivator in a given tissue might be sufficient to dictate the transactivation potential of these receptors. On the other hand, crystal structure studies also revealed that the LBD contains a flexible ligand-binding pocket that, although quite small (~100 Å) in the unliganded state (36, 38), can be rearranged to accommodate synthetic molecules that occupy much larger volumes (37, 39). In agreement with these ob-

servations, the presence of several synthetic molecules has been shown to inhibit the constitutive transcriptional activity of the ERRs. For example, the synthetic estrogen agonist diethylstilbestrol inhibits the activity of all three ERR isoforms, whereas the selective ER modulator 4-hydroxytamoxifen negatively affects the activity of only ERRβ and ERRγ (40–42). Compounds with better specificity against the ERRs have recently been identified. XCT790 acts as a specific inverse agonist for ERRα, whereas GSK5182, a derivative of 4-hydroxytamoxifen, displayed improved inverse agonist selectivity for ERRγ (43–45). Although the ERRs show constitutive transcriptional activity, the identification of two structurally related phenolic acyl hydrazone compounds, DY131 and GSK4716, with weak but specific agonist activity toward ERRβ and ERRγ has also been reported (46, 47). ERR transcriptional activity can thus be inhibited or stimulated by pharmacological agents. The presence of a functionally conserved LBD also indicates a high probability that the transcriptional activity of the ERRs is controlled *in vivo* by one or more natural ligands.

B. ERRs interact with nuclear receptor coregulators involved in metabolic control

The transcriptional activity of nuclear receptors is dependent on the presence of coregulatory proteins. More than 200 nuclear receptor coactivator and corepressor proteins have been identified to date, each one displaying different patterns of expression, enzymatic activity, and specificity of interactions with members of the superfamily of nuclear receptors as well as with other classes of transcription factors (48, 49). A subset of nuclear receptor coregulators have been shown to modulate specific metabolic programs (50). In particular, PPARγ coactivator (PGC)-1α and PGC-1β have been shown to play essential roles in mitochondrial biogenesis, fuel intake, FAO, adaptive thermogenesis, gluconeogenesis, circadian activity, and production of reactive oxygen species (ROS) (51–61). Genetic studies in mice have demonstrated that steroid receptor coactivator (SRC)-1, -2, and -3 regulate hepatic metabolism, fat storage, and energy balance (62–65). Similarly, the absence of the corepressor known as receptor interacting protein 140 (RIP140 or NRIP1) in mice leads to reduced body weight and fat content as well as an increase in oxidative metabolism in skeletal muscle (66–68). The *Nrip1*-null mice also display a higher glucose tolerance and insulin responsiveness when challenged with a high-fat diet (69). The three classes of coregulators have been shown to interact with one or more ERR isoform. SRC-1, -2, and -3 were first demonstrated to interact with the LBD of ERR and stimulate the transcriptional activity of ERRα and ERRβ when cointroduced in mammalian cells (70), whereas ERRγ was originally identified in a yeast two-hybrid screen aimed at cloning proteins interacting with SRC-2 (20). The ERRs also directly interact with PGC-1α and PGC-1β via their AF-2 domains and sequence-specific leucine-rich nuclear receptor interacting motifs (LxxLL) in the PGC-1 proteins (58, 71–75) (Fig. 1B). PGC-1α contains three distinct LXXLL-like motifs, but only motifs 2 and 3 have been shown to mediate the interaction with nuclear receptors. Motif 2 is recognized by a large number of nuclear receptors, including the PPARs, whereas motif 3, an atypical reverse LXXLL motif (LLKYL), interacts exclusively with the ERRs (71, 73). Indeed, the atypical LLKYL motif 3 and surrounding amino acid residues make specific contacts with the ERRα LBD that involve helices 4, 8, and 9, contacts that cannot be made with ERα (76). The specific allosteric role of helices 8 and 9 in ERRα/PGC-1α interaction may explain the previous observations that DNA binding and ERRα homodimerization affect PGC-1α binding to and the transcriptional activity of ERRα (27, 29, 77, 78). The activity of the ERRs as transcription factors is generally inhibited through physical interaction with RIP140, although activation resulting from this interaction has also been observed in ERR isoform- and promoter-specific manners (79–82). RIP140 contains nine LXXLL motifs and one atypical LXXML motif (Fig. 1C). The precise motifs responsible for interaction with the ERRs have not been delineated, but distinct regions in RIP140 have been shown to bind the ERRs. PGC-1α and RIP140 thus compete for interaction with the ERRs for positive and negative control of gene expression (Fig. 1D).

C. ERRs are expressed in tissues with high metabolic needs

At least one ERR isoform is expressed in every tissue examined to date (83). ERRα is ubiquitously expressed and found in more abundant quantities than the two other isoforms (Fig. 2). In particular, high levels of ERRα can be detected in tissues with high metabolic needs such as the heart, kidneys, intestinal tract, skeletal muscle, and brown adipose tissue (BAT) (17, 25, 83, 84). Indeed, anatomical profiling of nuclear receptor expression in 39 different tissues showed that ERRα segregated with tissues involved in lipid metabolism and energy homeostasis (83). ERRα is also expressed throughout the adipocyte differentiation program (85) and in bone-derived macrophages activated by lipopolysaccharide or interferon γ (IFN-γ) (58, 86). ERRβ and ERRγ show more restricted expression patterns and are, in general, expressed at lower levels than those of ERRα. However, both ERRβ and ERRγ are present in abundance in the heart and kidneys, and their expression patterns segregate with tissues associated with basal metabolic functions (83). All three ERR isoforms are widely expressed in the central

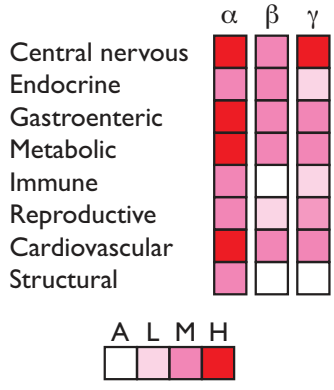

FIG. 2. Distribution of mRNA expression for ERRα, ERRβ, and ERRγ according to a functional grouping of tissues. The data are adapted from Bookout *et al.* (83). Levels of expression are represented as: A, absent; L, low; M, medium; H, high.

nervous system (87). In particular, ERRα and ERRγ can be found at high levels in the hypothalamus, an anatomical region of the brain known to play an important role in basic body homeostasis (87). Finally, light/dark-dependent oscillations in ERR isoform expression in key metabolic tissues suggest a role for these orphan receptors in linking the peripheral circadian clock with specific metabolic outputs (88). ERRβ was shown to be rhythmically expressed in the liver, BAT, white adipose tissue (WAT), and skeletal muscle (88). In contrast, ERRα and ERRγ expression selectively oscillates in WAT and liver and BAT and liver, respectively. When taken in combination, the expression of ERR isoforms in highly oxidative tissues and the cyclic behavior of ERR transcripts in metabolic tissues led to the suggestion that the ERRs may operate as molecular links between the circadian oscillator and energy metabolism (88).

Little is known about the mechanisms and signals governing the expression of the three ERRs. ERRα expression is stimulated by physiological stimuli such as exposure to cold temperatures, exercise, and fasting, in a pattern parallel to that of PGC-1α (73, 84, 89). Indeed, the *ESRRA* promoter encodes a PGC-1α/ERRα-responsive autoregulatory element that can also be recognized and activated by ERRβ and ERRγ (26, 72, 90, 91). Induction of PGC-1α expression by physiological stimuli is thus believed to increase the transcriptional activity of the ERRs, leading to higher expression of ERRα and other ERR target genes in a positive feedforward mechanism.

III. ERRs as Regulators of Energy Metabolism

In the absence of a natural ligand linking the ERRs to biological functions, identification of target genes initially offered a powerful alternative approach to identifying physiological processes under the transcriptional control of the ERRs. Indeed, the identification and characterization of the first ERR target genes helped establish the concept of ERRs as key regulators of energy metabolism.

A. Fatty acid oxidation

Medium-chain acyl-coenzyme A dehydrogenase (MCAD, *Acadm*) is an enzyme that mediates the first step in the mitochondrial β-oxidation of fatty acids (92). The levels of MCAD expression dictate the rate of tissue FAO and are thus closely regulated by tissue energy demands. Accordingly, tissues that preferentially utilize fatty acids as energy substrates such as the heart and kidneys express the highest MCAD levels (93). FAO also provides the reducing equivalents for the mitochondrial uncoupling reaction involved in the dissipation of energy as heat, and consequently high levels of MCAD can also be found in BAT (94). Initial studies on the regulatory pathways controlling the expression of MCAD led to the identification of a complex nuclear receptor response element referred to as NRRE-1, located in the proximal region of the promoter driving its expression (95–97). This element was shown to be essential for the proper expression of a reporter gene in the heart and BAT of transgenic mice and for the transcriptional induction of the gene during brown adipocyte differentiation *in vitro* (98). Although the MCAD NRRE-1 could confer transcriptional activation by the retinoic acid receptor (RAR)/retinoid X receptor (RXR) complex (99), PPARα (100), HNF-4α (97), or repression by COUP-TF I and II (96), the endogenous nuclear receptors responsible for modulation of MCAD expression in tissues with high energy needs were not known. As described above, identification of the ERRE as the consensus sequence TCAAGGTCA led to the realization that an ERRE was embedded within the NRRE-1 element and that, when isolated, the element could impart ERRα responsiveness to a heterologous promoter (25). Further experiments using an EMSA and a specific ERRα antibody demonstrated that endogenous ERRα, present in nuclear extracts prepared from brown adipocytes in culture, was the main nuclear protein binding to the NRRE-1 (25, 101). The same approach was later used to show that ERRα present in the liver, heart, and kidneys of mice also binds to the *Acadm* promoter (102). The recruitment of ERRα at the *Acadm* promoter *in vivo* was also confirmed by ChIP assays using chromatin obtained from mouse hearts (26). Introduction of PGC-1α, but not of a mutant protein lacking functional L2 and L3 motifs, induced MCAD expression, an effect also abolished by a small interfering RNA (siRNA) targeting ERRα (73). On the other hand, elevated expression of MCAD in myotubes devoid of RIP140 can be decreased by the ERRα inverse agonist XCT790 (68). A direct effect of ERRα on mitochondrial FAO was observed in rat neonatal cardiac myocytes and mouse embryonic fibroblasts devoid of ERRα (103). In both cell types, overexpression of ERRα led to a significant increase in palmitate oxidation. Taken together, the identification of ERRα as a transcriptional regulator of *Acadm*, the observation that ERRα and MCAD were coexpressed in tissues with high energy needs, and the direct effect of ERRα on FAO provided the initial evidence of a strong link between the metabolic potential of a tissue and the presence and transcriptional activity of ERRα.

B. Mitochondrial biogenesis and oxidative capacity

As described above, ERRα and PGC-1α are not only coexpressed in tissues with high energy demands, but PGC-1α also strongly activates the transcriptional activity of ERRα. Taken together, these observations led to the hypothesis that the regulation of key metabolic processes by PGC-1α, such as mitochondrial biogenesis and oxidative phosphorylation (OXPHOS), could be transduced by ERRα. Accordingly, Schreiber *et al.* (104) used an adenovirus to introduce PGC-1α in SAOS2 cells and monitored changes in gene expression by high-density oligonucleotide microarrays. Expression of PGC-1α was associated with the up-regulation of 151 genes encoding mitochondrial proteins involved in FAO, the tricarboxylic acid cycle (TCA), and OXPHOS, as well as in the mitochondrial ribosomal machinery and transport across the mitochondrial membrane. PGC-1α induction of genes encoding mitochondrial proteins was accompanied by an increase in mitochondrial DNA content, an effect that could either be blocked by an siRNA against ERRα or mimicked by the introduction of a transcriptionally constitutive ERRα-VP16 chimeric construct. These tools were also used to validate the requirement for ERRα in the induction of several

nuclear-encoded mitochondrial genes by PGC-1α, including *Cycs*, *Atp5b*, and *Idh3a*.

Using a similar approach but applying different diagnostic tools to analyze the data, Mootha *et al.* (90) performed expression profiling of C2C12 myoblast cells differentiated into myotubes and transduced with an adenovirus expressing PGC-1α. A large proportion of the 649 genes up-regulated by PGC-1α were also linked to carbohydrate metabolism and mitochondrial functions. The use of a novel motif-finding algorithm led to the discovery that a significant number of promoters contained binding sites for ERRα. Treatment of C2C12 cells with the ERRα inverse agonist XCT790 abolished the activity of the ERRα/PGC-1α complex on a subset of promoters containing an ERRE, diminished the PGC-1α induction of MCAD expression, and reduced total mitochondrial respiration induced by PGC-1α. An extended role for ERRα in mitochondrial biogenesis had also been previously proposed by Mootha *et al.* (105) based on their analysis of a large-scale proteomic survey of mitochondrial proteins and integration of this data set with known gene expression profiles. In that study, it was observed that ERRα expression was tightly correlated with genes whose products reside in or are in close association with the mitochondrion.

The strategy to use PGC-1α as a surrogate ligand to activate endogenous ERRs was refined further by engineering a PGC-1α mutant that specifically recognizes ERRα (106). Introduction of this PGC-1α mutant in HepG2 cells and subsequent analysis of the modified gene expression profile confirmed that ERRα is a key regulator of OXPHOS and other related energy metabolism pathways. Adenovirus-mediated PGC-1α expression in mouse embryonic fibroblasts (MEFs) derived from wild-type and ERRα-null mice was also used to assess ERRα-dependent gene expression profiles (107). As previously observed, genes involved in mitochondrial function were among transcripts differentially expressed in ERRα-null cells. In addition, it was shown that PGC-1α failed to induce citrate synthase activity, a key indicator of mitochondrial activity, in the absence of ERRα.

Genome-wide location analysis of ERRα and ERRγ binding sites in proximal promoters also led to the identification of a large number of genes under potential regulation by the ERRs (26, 58, 108). The ERR-bound regions were identified using a custom-made mouse genomic DNA array containing the region spanning 800 base pairs upstream and 200 base pairs downstream of transcriptional start sites of approximately 19,000 genes. Gene ontology analysis of the genes associated with ERR-bound promoters revealed that a significantly large number of these genes encode mitochondrial proteins (Table 1). This gene list included previously validated ERR targets such as *Acadm*, *Atp5b*, *Cycs*, and *Idh3a*, whereas a direct role for ERRs in the regulation of other genes such as *Aco2*, *Mtch2*, and *Timm8b* was shown in these studies, suggesting that the vast majority of ERR binding sites found in proximal promoters are functional. A survey of the list of ERR target genes (Table 1) shows that the ERRs may control most aspects of mitochondrial functions, including FAO (*e.g.*, *Acadm*, *Mylcd*), the TCA cycle (*e.g.*, *Fh1*, *Sdhd*), OXPHOS (*e.g.*, *Cycs*, *Ndfus1*), ATP production (*e.g.*, *Atp5b*, *Atp5g3*), and export (*e.g.*, *Slc25a4*, encoding Ant1), as well as transcription of mitochondrial genome-encoded genes (*e.g.*, *Tfb2m*) and the translation apparatus (*e.g.*, *Mrpl19*) (Fig. 3). Unpublished work from our laboratory using promoter arrays covering as much as 8 kilobases of DNA centered around the transcriptional start sites of genes shows that the ERRs may directly regulate the expression of close to half of the mitochondrial proteome. ERRα is also involved in the control of the biogenesis and maintenance of the mitochondrial network though regulation of the levels of mitofusin 1 and 2 (89, 109). The mitofusins are mitochondrial membrane proteins that participate in mitochondrial fusion and contribute to the maintenance and operation of the organelle. The *MFN2* promoter contains a conserved ERRE and is activated by ERRα in the presence of PGC-1α (89).

The studies cited above clearly established a direct involvement of ERRα in mitochondrial biogenesis. ERRα, working in concert with ERRγ, responds to induced levels of PGC-1α by up-regulating the expression of other factors such as PPARα and GA binding protein α (GABPA), as well as its own (Fig. 4). However, the observation that ERRα-null mice are viable and MEFs derived from these animals display normal growth and proliferation demonstrates that ERRα by itself is not absolutely essential for this process (72, 110). In addition, it was shown that overexpression of PGC-1α in ERRα-null MEFs could still induce FAO in those cells, suggesting that another PGC-1α partner is able to promote mitochondrial functions (103). In fact, PGC-1α itself is not absolutely essential for mitochondrial biogenesis (57). Because ERRα and ERRγ (and most probably ERRβ) can share the same target genes (26), it is likely that genetic and functional redundancies between ERR isoforms are responsible for the lack of an overt mitochondrial phenotype in the absence of ERRα.

C. Glucose metabolism

Cellular glucose utilization is tightly controlled. One key step in this process is the regulation of pyruvate entry into the TCA cycle by the pyruvate dehydrogenase complex (PDC). The PDC, a large molecule containing 60 subunits of three different enzymes, catalyzes the irreversible decarboxylation of pyruvate to acetyl coenzyme A (CoA). The activity of the PDC is controlled by both allosteric regulation of fatty acid intermediates of FAO and posttranslational modifications. In particular, PDC can be reversibly phosphorylated by members of the pyruvate dehydrogenase kinase (PDK) 1–4 family, a modification that inactivates the enzymatic complex. Inhibition of the PDC thus supports a switch from glucose oxidation to FAO. Because glucose oxidation and FAO pathways can be controlled by shared regulatory mechanisms, Wende *et al.* (111) explored the possibility that PGC-1α could influence glucose oxidation via transcriptional control of PDK4 expression. Indeed, introduction of PGC-1α in C2C12 myotubes was shown to induce its expression. Surprisingly, neither PPARα nor forkhead box O1 (FOXO1), two transcription factors previously implicated in the regulation of PDK4 and known partners of PGC-1α, were required for the induction. Rather, PGC-1α was shown to activate the *Pdk4* promoter via a nuclear receptor binding site occupied by ERRα, whereas the PGC-1α effect was lost in MEFs derived from ERRα-null mice. Similarly, introduction

TABLE 1. Genes encoding mitochondrial proteins whose proximal promoter regions are bound by ERRα and/or ERRγ

Gene	Official full name	Function	ERRE	Position	Dir	Ref.
Acadm	Acetyl-CoA dehydrogenase, medium chain	Fatty acid oxidation	TAAAGGTCA	−557	F	25, 26, 44, 90, 101, 102, 103, 104, 106, 108, 110, 151, 158
Aco2	Aconitase 2, mitochondrial	TCA	TCAAGGTCA	−225	R	26, 58, 104, 106, 108, 151
Atp5b	ATP synthase, H+ transporting mitochondrial F1 complex, β−subunit	ATP synthesis	CCAAGGACA	−325	F	26, 58, 106,108, 151, 158
			TCAAGGTCA	+198	R	
Atp5c1	ATP synthase, H+ transporting, mitochondrial F1 complex, γ polypeptide 1	ATP synthesis	ACAAGGTCA	+246	F	26, 58, 104
Atp5d	ATP synthase, H+ transporting, mitochondrial F1 complex, δ subunit	ATP synthesis	CACGGGTCA	+291	R	58
			ATTAGGTCA	+308	F	
Atp5f1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	ATP synthesis	TCGGGGTCA	−1	F	26
			ACAGGGACA	+7	F	
Atp5g1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	ATP synthesis	AGAAGGACA	−208	R	26, 104
			AGAAGGACA	−156	R	
			AAAAGGTCA	+267	R	
Atp5g3	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 3	ATP synthesis	TCAAGGTCA	−75	R	26, 58, 104
			TTAAGGTCA	+215	R	
Atp5k	ATP synthase, H+ transporting, mitochondrial F1F0 complex, subunit e	ATP synthesis	CGAAGGTCA	+1	F	58
Cabc1	Chaperone, ABC1 activity of bc1 complex homolog	Electron transfer	TTAAGGACA	−48	F	26
Ccdc90b	Coiled-coil domain containing 90B	Unknown	CTAGGGACA	−792	R	58
			CGCAGGTCA	−7	R	
Ckmt2	Creatine kinase, mitochondrial 2	Phosphocreatine metabolism	TCAAGGTCA	+60	F	26, 101, 118, 110, 158
			TAAAGGACA	+97	F	
Clybl	Citrate lyase β like	Hydrocarbon metabolism	CAGAGGTCA	+176	R	26, 104, 108
Coq7	Demethyl-Q 7	Ubiquinone biosynthesis	TCAGGGTGA	+9	R	26, 58, 104, 108
			TGGAGGTCA	+195	F	
Cox5b	Cytochrome c oxidase, subunit Vb	Respiratory chain	TAAAGGTCA	−553	F	58, 90, 108
			CCAAGGTCA	+353	R	
Cox6c	Cytochrome c oxidase, subunit VIc	Respiratory chain	TCAAGGTCA	+440	R	26, 104, 108
Cox7a2	Cytochrome c oxidase, subunit VIIa 2	Respiratory chain	GGAGGGTCA	+258	F	58
Cox7b	Cytochrome c oxidase subunit VIIb	Respiratory chain	TATAGGTCA	−26	F	106, 108
			ACAAGGACA	+371	R	
Cox7c	Cytochrome c oxidase subunit VIIc	Respiratory chain	TGAAGGTCA	+413	F	108, 153
Cox8a	Cytochrome c oxidase, subunit VIIIa	Respiratory chain	TCAAGGTCA	−834	F	26, 108
			CCAAGGTCA	+71	R	
Cox8b	Cytochrome c oxidase, subunit VIIIb	Respiratory chain	AGAAGGACA	−872	F	26, 103, 104, 158
			AGAAGGACA	−664	F	
Cpt1b	Carnitine palmitoyltransferase 1b, muscle	Fatty acid metabolism		−754		108, 151, 158
Cpt2	Carnitine palmitoyltransferase 2	Fatty acid metabolism	TAGAGGACA	−896	F	26, 108
Cycs	Cytochrome c, somatic	Respiratory chain	TAAAGGTCA	−494	F	26, 58, 103, 104, 106, 108, 110, 151, 158
Cyp27a1	Cytochrome P450, family 27, subfamily A, polypeptide 1	Cholesterol metabolism	TGAGGGACA	−363	F	26, 108
			TGCAGGTCA	−339	F	
D10Ertd322e	DNA segment, Chr 10, ERATO Doi 322, expressed	Translation	GCAAGGTCA	+236	R	26
Endog	Endonuclease G	DNA metabolism	CTAGGGTCA	−307	R	26
			CTAGGGTCA	+82	R	
Etfb	Electron-transfer-flavoprotein, β polypeptide	Fatty acid catabolism	AGAGGGTCA	+37	F	26, 104, 108
			GTCGGGTCA	+188	R	
Etfdh	Electron-transferring-flavoprotein dehydrogenase	Fatty acid catabolism	TCCGGGTCA	−69	R	26, 103, 104, 106, 158
			CAAAGGTCA	+326	F	
Fh1	Fumarate hydratase 1	Tricarboxylic acid cycle	TGAAGGTCA	+172	R	26, 104
Got2	Glutamate oxaloacetate transaminase 2, mitochondrial	Amino acid metabolism	ACAGGGACA	−451	F	26, 58
			CTCGGGTCA	−345	F	
			CGGAGGTCA	−162	R	
Hdhd3	Haloacid dehalogenase-like hydrolase domain containing 3	Metabolism	TTAAGGTCA	−195	R	26
Hk2	Hexokinase 2	Glucose metabolism	CCAAGGTCA	−587	R	26, 158
			ACTGGGTCA	+177	R	
Hspa9a	Heat shock protein 9	Protein folding	TCAAGGTCA	−292	R	58
Idh3a	Isocitrate dehydrogenase 3 (NAD+) α	Tricarboxylic acid cycle	TCAAGGTCA	+221	F	58, 104, 106
			TGAGGGTCA	+304	F	
Isca1	Iron-sulfur cluster assembly 1 homolog (*S. cerevisiae*)	Respiratory chain	GCAAGGTCA	−141	F	58
			CACAGGTCA	−133	F	
			GAAAGGTCA	+354	F	

TABLE 1. —continued

Gene	Official full name	Function	ERRE	Position	Dir	Ref.
Iscu	Iron-sulfur cluster scaffold homolog (*E. coli*)	Respiratory chain	GGAAGGTCA	−845	F	26
Me3	Malic enzyme 3, NADP(+)-dependent, mitochondrial	Tricarboxylic acid cycle	TGAGGGTCA	−559	R	26, 104
			CCAAGGTCA	+61	R	
Mtx2	Metaxin 2	Protein transport	CCAAGGTCA	+377	F	26
Mrpl11	Mitochondrial ribosomal protein L11	Translation	TTTGGGTCA	+14	R	58
			ACAAGGACA	+314	R	
Mrpl19	Mitochondrial ribosomal protein L19	Translation	TTTAGGTCA	−546	R	58
Mrpl34	Mitochondrial ribosomal protein L34	Translation	TCCGGGTCA	+1	R	26
			GGCGGGTCA	+22	R	
Mrpl47	Mitochondrial ribosomal protein L47	Translation	CCAAGGTCA	−490	F	58
			ACGAGGTCA	+4	R	
			GCAAGGTCA	+239	R	
Mrps18b	Mitochondrial ribosomal protein S18B	Translation	GCCGGGACA	−537	F	26
Mtch2	Mitochondrial carrier homolog 2	Transport	GCAAGGTCA	−188	R	26, 103, 104
			TCAGGGTCA	−179	F	
Mfn2	Mitofusin 2	Mitochondrial fusion	TGAAGGACA	−559	F	89, 109
			ACAGGGTCA	−461	F	
Mlycd	Malonyl-CoA decarboxylase	Fatty acid oxidation	TCAAGGTCA	+923	F	26
Ndufa4	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 4	Respiratory chain	CCAGGGTCA	−284	R	26, 104
Ndufa5	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 5	Respiratory chain	ATGAGGTCA	−387	R	58
			TGGAGGTCA	+172	F	
Ndufa8	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 8	Respiratory chain	TCAAGGTCA	−980	R	26, 104
Ndufa9	NADH dehydrogenase (ubiquinone) 1 α subcomplex, 9	Respiratory chain	AAAGGGTCT	−301	F	26, 58, 104
			GCGGGGTCA	−38	R	
			GAAAGGTCA	+122	R	
Ndufb2	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 2	Respiratory chain	GTAAGGTCA	−200	F	26
			CAGGGGACA	−73	R	
Ndufb4	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 4	Respiratory chain	TCAAGGTCA	−436	F	26, 58, 104
			TCAAGGTCA	−379	R	
			TGAAGGTCA	−56	F	
Ndufb5	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 5	Respiratory chain	GCAAGGTCA	−525	F	26, 58, 104, 108
			ACGAGGTCA	−290	F	
			CCAAGGTCA	+204	R	
Ndufs1	NADH dehydrogenase (ubiquinone) Fe-S protein 1	Respiratory chain	TAAAGGTCA	−349	F	26, 58, 104, 108
			GAGGGGTCA	+66	F	
			CATAGGTCA	+196	F	
Ndufs2	NADH dehydrogenase (ubiquinone) Fe-S protein 2	Respiratory chain	GGGAGGTCA	−91	R	26
			CAGGGGTCA	+206	R	
			CCTGGGTCA	+224	R	
Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	Respiratory chain	GCACGGTCA	−336	R	58, 104, 158
			GCAAGGACA	+378	R	
			GGAAGGACA	+394	R	
			CCAAGGACA	+422	R	
Ndufs7	NADH dehydrogenase (ubiquinone) Fe-S protein 7	Respiratory chain	AGCGGGTCA	−96	F	26, 58, 108
			TATAGGTCA	+154	F	
Ndufv1	NADH dehydrogenase (ubiquinone) flavoprotein 1	Respiratory chain	CCAGGCTCA	−186	R	26
			GACGGGTCA	+65	F	
Pdk4	Pyruvate dehydrogenase kinase, isozyme 4	Glucose metabolism	ACAAGGACA	−360	F	26, 104, 110, 111, 112, 113
Rtn4ip1	Reticulon 4 interacting protein 1	Metabolism	CCCGGGTCA	−263	R	26
			CCTAGGTCA	+74	R	
Sdha	Succinate dehydrogenase complex, subunit A, flavoprotein	Tricarboxylic acid cycle	CGAAGGTCA	+202	F	26, 58, 106, 158
Sdhb	Succinate dehydrogenase complex, subunit B, iron sulfur	Tricarboxylic acid cycle	CAGAGGTCA	−88	F	26, 58, 104, 106, 158
Sdhd	Succinate dehydrogenase complex, subunit D, integral membrane protein	Tricarboxylic acid cycle	TCAAGGTCA	+13	R	26, 58, 104, 106, 108
Slc25a4	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 4	Transport	TCGAGGTCA	−391	F	26, 104, 106, 108, 158
			AGAGGGTCA	+176	F	
			TGCAGGTCA	+194		
Slc25a29	Solute carrier family 25 (mitochondrial carrier, palmitoylcarnitine transporter), member 29	Transport	TCAACCTCA	−327	R	104, 108
			GAAGGGTCA	+297	R	
Suclg1	Succinate-CoA ligase, GDP-forming, α-subunit	Tricarboxylic acid cycle	TGTGGGACA	−240	R	58, 104, 106

TABLE 1. —continued

Gene	Official full name	Function	ERRE	Position	Dir	Ref.
Supv3l1	Suppressor of var1, 3-like 1	Helicase	GAATGGTCA	−767	F	58
Tfb2m	Transcription factor B2, mitochondrial	Transcription	GGGAGGACA	−446	F	58
Timm8b	Translocase of inner mitochondrial membrane 8 homolog b	Protein transport	TCAAGGTCA	−104	F	26
Timm9	Translocase of inner mitochondrial membrane 9 homolog	Protein transport	GGAGGGTCA	+50	R	58
			TCAAGGTCA	+410	R	
Timm10	Translocase of inner mitochondrial membrane 10 homolog	Protein transport	TCTAGGTCA	−585	F	58
			TTAGGGTCA	+214	F	
			TCAAGGTCA	+366	R	
Timm17a	Translocase of inner mitochondrial membrane 17 homolog a	Protein transport	TAAGGGACA	+74	R	26
			ACAAGGTCA	+233	R	
Txn2	Thioredoxin 2	Redox homeostasis	CTAGCGTCA	+2	R	104
1110020P15Rik	RIKEN cDNA 1110020P15 gene	Electron transfer	CCAAGGTCA	+200	R	58

Gene names in *bold* indicate that, in addition to ERRα and/or ERRγ binding to the promoter region, modulation of ERRα and/or ERRγ activity in cultured cells or *in vivo* was shown to modulate the expression of that gene. Dir, Direction; F, forward; R, reverse.

of an siRNA against ERRα in CV-1 cells prevented PGC-1α-dependent induction of the *Pdk4* promoter (112). A third study showed that both ERRα and ERRγ can activate the rat PDK4 gene promoter in hepatoma cells and that although FOXO1 was not directly involved in the response to PGC-1, it could be needed for the synergistic effects of ERRα and PGC-1α (113). Thus, through the regulation of PDK4 expression, the ERRα/PGC-1α complex is also involved in the regulation of energy substrate selection.

ERRα has also been shown to repress the gluconeogenic action of PGC-1α via its effects on the promoter of the gene encoding PEPCK (84, 114). PEPCK is a key rate-limiting enzyme in hepatic gluconeogenesis that is active during periods of fasting. The expression of PEPCK is under intense hormonal regulation that involves insulin, glucagon, and glucocorticoids. The effect of these hormones is transduced via a number of transcription factors that include CCAAT/enhancer binding proteins, cAMP-response element-binding protein, AP-1, FOXO1, and the glucocorticoid receptor as well as the orphan nuclear receptors HNF-4α and COUP-TF1. PGC-1α has been shown to induce PEPCK expression through interactions with HNF-4α, FOXO1, and the glucocorticoid receptor (115–118). In contrast, ERRα, via binding to an ERRE located in the *PEPCK* promoter, appears to interfere with the recruitment of PGC-1α to the proximal regulatory region of *PEPCK* (114). This effect is gene specific because induction of mitochondrial genes involved in the process of OXPHOS by PGC-1α was still dependent on the presence of ERRα. Although PEPCK expression was found to be elevated in the liver of fed ERRα-null mice, blood glucose levels were normal in the fed state. This last observation highlights the complexity of the regulatory pathways controlling energy homeostasis in the whole animal and the specific roles that ERRα may play in each tissue.

D. Transcriptional control of metabolism

The ERRs not only control the expression of genes whose products are directly involved in cellular energy homeostasis but also regulate the expression of transcription factors that, on their own, can influence this process. In that capacity, the ERRs are thus initiators of extensive transcriptional regulatory networks implicated in the control of energy metabolism.

1. Nuclear receptors. As mentioned in *Section* II.B.C., ERRα can control its own expression via binding to a conserved regulatory region located in the promoter of the *ESRRA* gene (72, 90). Binding of both ERRα and ERRγ has been observed at this site using ChIP-based techniques, and the two receptor isoforms can activate the *ESRRA* promoter in transient transfection assays (26, 91). Interestingly, this regulatory region is polymorphic in humans and contains one to four copies of an ERRE, in addition to another ERRE located outside of the polymorphic sequence (72, 119). The transcriptional response of the *ESRRA* promoter to the presence of ERRs and PGC-1α is directly proportional to the number of ERREs contained in the promoter, suggesting that this polymorphism may lead to differential responses to ERR/PGC-1-mediated metabolic cues in the human population (72).

Thyroid hormone is an important regulator of thermogenesis, cardiac function, and overall maintenance of energy homeostasis. In peripheral tissues, thyroid hormone can increase free fatty acid and glucose levels by enhancing lipid mobilization as well as gluconeogenesis and glycogenolysis. The action of thyroid hormone in the sympathetic nervous system is essential for adaptive thermogenesis and the adrenergic response of the WAT. Thyroid hormone regulates gene expression through binding to two members of the nuclear receptor superfamily, thyroid hormone receptor (TR) α (NR1A1) and TRβ (NR1A2) (120). The two receptors act as strong transcriptional repressors in the absence of the hor-

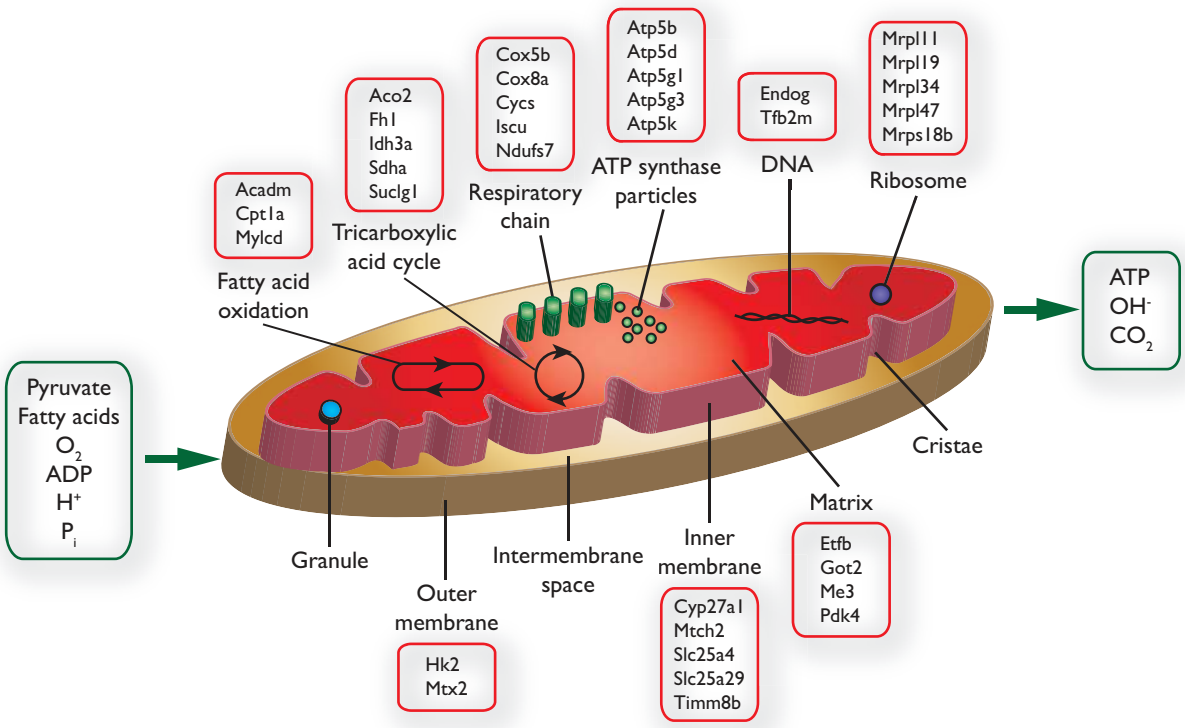

FIG. 3. ERRs regulate nuclear-encoded genes whose products are involved in all aspects of mitochondrial functions. Examples of genes whose promoters are bound by ERRα and ERRγ are shown in *red boxes* according to functions or location within the mitochondrion. Metabolites and molecules involved in the generation of energy are shown within *green boxes*.

mone and as activators of gene expression in the liganded form when bound to positive thyroid hormone response elements. In particular, studies in knockout mice have shown that TRα is required for normal thermogenesis and heart function and is essential for metabolic homeostasis (121–126). Because TRα and TRβ can actively regulate gene expression even in the absence of thyroid hormone, knowledge about their expression levels and factors regulating them in target tissues is particularly important. The promoter driving the expression of the TRα1 isoform contains a perfect ERRE at position −443, which was shown to be both necessary and sufficient to mediate transcriptional activation of the promoter by ERRα (127). A subsequent study also showed that transient transfection of all three ERR isoforms leads to an increase in the activity of the TRα promoter (80). However, deletion of the ERRE at position −443 did not significantly affect the response to the three ERRs, suggesting that ERR regulation of the TRα promoter might involve other sites and mechanisms (80). TRα1 and ERRα are both coexpressed in several tissues in which they are active, including intestine, BAT, and heart, suggesting that transcriptional control of TRα1 expression by ERRα might be physiologically relevant. It should be noted that endogenous TRα1 expression has yet to be reported as altered in an ERR knockout mouse model.

Like ERRα, PPARα is highly expressed in tissues with high energy demands. PPARα has been implicated in the control of lipid, lipoprotein, and glucose metabolism, functions that are shared with ERRα (128). As observed with MCAD, the two receptors can target the same gene for transcriptional regulation. Another reason that the biological functions of the two receptors often overlap is that ERRα has been shown to directly regulate the expression of PPARα (103). Overexpression of ERRα in C2C12 skeletal myotubes leads to enhanced levels of PPARα, and the PPARα promoter contains a functional ERRE that can be activated by ERRα. Furthermore, the presence of PPARα is required for ERRα-mediated regulation of a subset of FAO targets in MEFs. Thus, in addition to directly regulating genes involved in mitochondrial biogenesis and FAO, ERRα also acts upstream of PPARα to enhance the control of shared target genes and indirectly by modulating genes more specific to the PPARα pathway.

ERRα and/or ERRγ have also been shown to bind the promoter regions of RARα (NR1B1) and RXRβ (NR1B2) (26, 58). Retinoic acid is a pleiotropic regulator of development and physiology and a potent modulator of cell growth and differentiation. As a heterodimer with RXR, RARα has been shown to regulate metabolic genes such as MCAD (25, 99), but its influence on metabolism also resides in its broad effects on the differentiation and survival of several cell types, including adipocytes (129, 130). By controlling the expression of RXRβ, ERRα has the potential to indirectly influence the activity of a large number of RXR partners involved in metabolic control (131). In addition to the RARs, the RXRs act as obligatory partners for numerous nuclear receptors implicated in energy homeostasis, including PPARα, -δ, and -γ, TRα and -γ, LXR, and FXR. RXRβ is ubiquitously expressed but is found at particularly high lev-

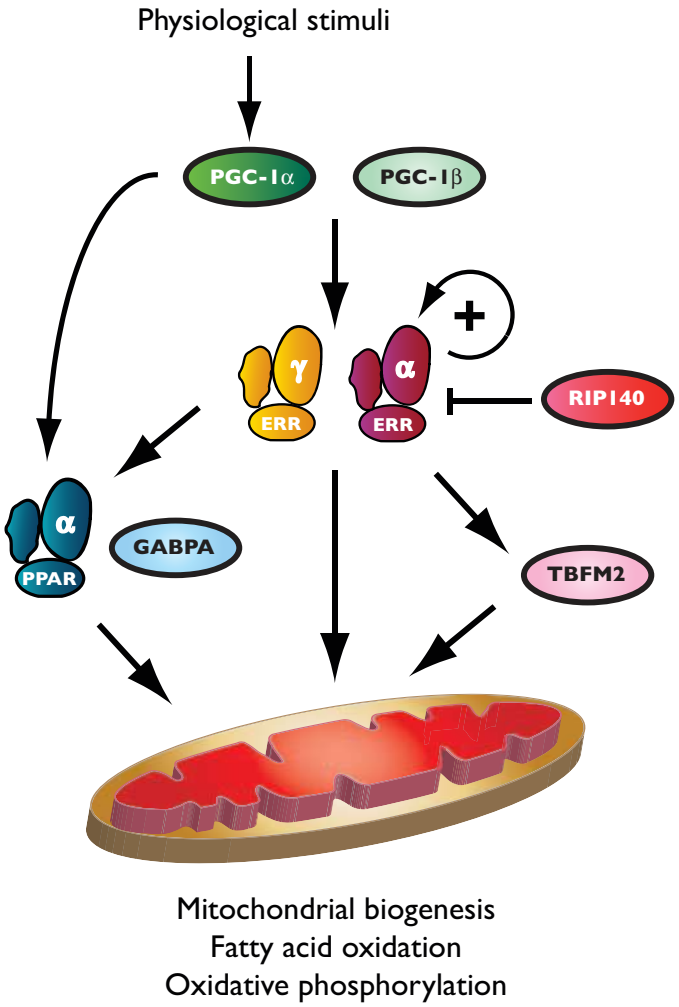

FIG. 4. A model of the ERR-based transcription network controlling mitochondrial biogenesis and function. The transcriptional activity of ERRα and ERRγ (and presumably ERRβ) is enhanced by constitutively present PGC-1β or by PGC-1α whose expression is turned on after physiological stimuli such as fasting, exercise, or exposure to cold temperatures. The expression of ERRα is then enhanced via an autoregulatory mechanism. ERRα and ERRγ then activate the expression of other transcription factors such as GABPA, PPARα, and TBFM2, and together all these factors activate vast gene networks involved in mitochondrial biogenesis and function. RIP140 whose expression is controlled by the ERRs may then participate in a negative regulatory loop to stop the initial ERR/PGC-1 response.

els in metabolic tissues and the cardiovascular system (83). Although it remains to be shown that binding of ERRα and ERRγ to the RARα and RXRβ promoters leads to the regulation of the cognate genes, control of crucial components of the retinoid/rexinoid pathway would significantly increase the range of control that the ERRs exert on energy metabolism.

The orphan nuclear receptor small heterodimer partner (SHP, NR0B2) is an atypical nuclear receptor lacking a DBD. SHP interacts with numerous nuclear receptors, including ERRα, ERRβ, and ERRγ, and inhibits their activity via a variety of mechanisms (132–135). Through its interaction with nuclear receptors and other transcription factors, SHP has been shown to modulate the expression of a large number of genes involved in metabolic processes. In particular, SHP appears to play important roles in bile acid metabolism, lipogenesis, glucose homeostasis, and steroid biosynthesis (132, 136–138). SHP has also been described as a negative regulator of energy production in BAT (134). In brown adipocytes, SHP inhibits the expression of PGC-1α via interference with the potent transcriptional activity of ERRγ on the PGC-1α promoter. SHP expression is positively regulated by ERRγ via binding to an atypical ERRE but apparently not by ERRα and ERRβ (133). Thus, stimulation of the expression of the transcriptional repressor SHP by the ERRs provides an inhibitory feedback mechanism to control for the initial feed-forward-positive PGC-1α/ERR loop.

2. RIP140. As a transcriptional repressor of nuclear receptors, RIP140 plays a crucial role in metabolic regulation and skeletal muscle function (66, 68). In adipocytes, RIP140 suppresses the expression of genes involved in FAO, glucose homeostasis, and energy expenditures, and many of these genes are direct targets of ERRα (69, 139). As observed with the transcriptional repressor SHP, ERRα stimulates the expression of RIP140, thus subjecting itself to feedback inhibition (140). ERRα enhances RIP140 expression via an alternative promoter (P2) that is preferentially used during adipogenesis. ERRα activates the P2 promoter by two different mechanisms involving direct binding to an ERRE at position −650 and indirect binding through Sp1 at a GC-rich element located in the proximal promoter region. Deleting the ERRE led to a loss of responsiveness to PGC-1α but not to ERRα, indicating that PGC-1α was not required for induction of gene expression by ERRα at the Sp1 site. These results suggest that the sensitivity of the feedback inhibition of ERRα activity via increased expression of RIP140 may depend on the cell-specific levels of PGC-1α and Sp1.

3. GABPA. GABPA is the DNA binding α-subunit of GABP, also known as nuclear respiratory factor-2 (NRF-2). GABPA binds DNA in the form of a heterotetramer (α2β2) with GABPB, the non-DNA binding β-subunit (141). NRF-2 directly regulates the expression of numerous mitochondrial proteins of the respiratory chain or indirectly via induction of transcription factors controlling their expression. GABPA binding sites were found to be particularly enriched in the promoters of genes encoding mitochondrial proteins and whose levels of expression were induced in the presence of PGC-1α in C2C12 myotubes (90). In this same study, it was found that the expression of both GABPA and ERRα was also induced by PGC-1α. The GABPA promoter was shown to contain a functional binding site for ERRα and, concurrently, inhibition of ERRα transcriptional activity by the inverse agonist XCT790 abolished the PGC-1α-dependent induction of GABPA in C2C12 cells. Recent genome-wide ChIP-on-chip experiments not only confirmed that the GABPA promoter is a *bona fide* ERRα target *in vivo* but also identified the promoter of GABPB as an ERRα-bound region (26, 58, 108). Thus, NRF-2 and ERRα are components of a double-positive feedback loop initiated by the presence of PGC-1α that plays an important role in mitochondrial gene expression and function.

4. TRP53. The tumor suppressor transformation-related protein 53 (TRP53 or p53) is known for its major role in the control of DNA repair, cell-cycle arrest, and cell death (142, 143). However, p53 is now emerging as a key regulator of genes involved in modulating mitochondrial respiration and setting the levels of ROS (144–147). It is therefore likely that p53 levels would be regulated by transcription factors known to regulate these processes. The expression of p53 is indeed down-regulated in the heart of ERRα-null mice, and both ERRα and ERRγ bind to the *Trp53* promoter *in vivo* as detected by ChIP-on-chip and standard ChIP experiments (26).

IV. Tissue-Specific Metabolic Phenotypes of ERR-Null Mice

Knockout mice for all three ERR isoforms have been generated, and the phenotypes resulting from ablation of those genes have been analyzed (108, 110, 148, 149). These mouse models cover the whole phenotypic spectrum, from embryonic lethal for the ERRβ-null mice and death shortly after birth for the ERRγ-null mice to viability and fertility for the ERRα-null animals. Although phenotypic analyses of the knockout mice showed that individual ERR isoforms are not absolutely essential for mitochondrial biogenesis *in vivo*, these mice displayed metabolic phenotypes that are tissue- and function-specific, and that are often essential for the survival of the organism when challenged with physiological stresses (Table 2).

A. Abnormal lipogenesis in white adipose tissue

Genetic ablation of *Esrra* resulted in ERRα-null mice that are viable and fertile with no gross anatomical alterations when housed under controlled laboratory conditions (110). The only readily apparent phenotype was reduced body weight and peripheral fat deposits. A straightforward explanation for the observed phenotype could not be obtained because no significant changes in food consumption, energy expenditure, or blood chemistry could be observed in the ERRα-null mice. However, the ERRα-null mice were also resistant to a high-fat diet-induced obesity, suggesting a complex role for ERRα in lipid metabolism at the level of the whole organism. It should be noted that these two phenotypes are not consistent with a decrease in oxidative metabolism as anticipated by the role played by ERRα as a transcriptional activator of oxidative metabolism genes. A similar inconsistent phenotype was also observed in the PGC-1α-null mice (57), suggesting a more intricate role for the ERRα/PGC-1α complex in the systematic regulation of energy homeostasis. In fact, the expression of the energy expenditure-promoting gene *Ucp1* was shown to be increased in WAT of ERRα-null mice. It is currently unknown whether ERRα-null adipocytes exhibit an increase in respiration. Nonetheless, gene expression profiling in ERRα-null WAT also showed alterations in the expression of genes involved in lipid metabolism and energy metabolism, including a reduction in the expression of *Fasn*, the gene encoding fatty acid synthase. Accordingly, the WAT of ERRα-null mice displayed significantly decreased lipogenesis in comparison to WAT of littermate controls. Thus, the initial phenotypic analysis of the ERRα-null mouse demonstrated that ERRα was essential for normal lipid metabolism and control of metabolic genes under basal conditions. Although the basal phenotype of the ERRα-null mice is relatively mild, the availability of viable ERRα-null animals allows for the investigation of the roles played by ERRα in the response to diverse physiological stresses.

B. Brown adipose tissue and intolerance to cold temperatures

The main function of BAT is to generate heat in response to cold temperatures via adaptive thermogenesis and is, thus, essential for an active life and survival in a cold-unprotected environment (150). As indicated above, ERRα is highly expressed in BAT, and the consequences of the absence of ERRα on BAT function were thus investigated (151). The BAT of ERRα-null mice was shown to have a decreased mitochondrial mass and a reduced oxidative capacity, an observation that reinforced the crucial role that ERRα plays in this process in BAT and other oxidative tissues. The ERRα-null mice were found to be defective in nonshivering thermogenesis and thus unable to maintain proper body temperature even when exposed to moderately low temperatures. However, the inability of ERRα-null mice to maintain body temperature was not a consequence of a lack of induction of genes involved in the thermogenic program. The levels of induction of *Ucp1* and *Dio2* expression were normal in the BAT of ERRα-null

TABLE 2. Tissue-specific phenotypes observed in ERR-null mice

Isoform	Tissue	Phenotype	Ref.
ERRα	BAT	Impaired mitochondrial biogenesis and thermogenesis	151
	Heart	Abnormal bioenergetic and functional adaptation to hemodynamic stressors	158
	Intestine	Lipid malabsorption	153
	Macrophages	Reduced ROS production and clearance of bacteria in response to IFN-γ	58
	WAT	Reduced lipogenesis and fat content	110
ERRβ	Placenta	Abnormal differentiation of the trophoblast lineage	149
	Inner ear	Alteration in the developmental fate of epithelial cells; defect in inner-ear fluid homeostasis	148
ERRγ	Heart	Electrocardiographic abnormalities; failure to switch to an oxidative gene program; increased mitochondrial biogenesis	108

mice upon exposure to cold, suggesting that ERRα is not directly involved in the transcriptional regulation of these genes in BAT. TRα is a more likely candidate as a regulator of the cold-induced thermogenic program, but BAT also expresses a high level of ERRγ, which could compensate for the absence of ERRα in regulating this specific gene network. In contrast, the expression of mitochondrial genes involved in energy metabolism was affected in BAT of ERRα-null mice. Together, the study of the role played by ERRα in BAT indicated once again that, in the absence of ERRα, the mitochondrial capacity is sufficient to maintain basal mitochondrial function in oxidative tissues, but that stressors that increase energy demand exceed the inherent capacity of the ERRα-null mitochondria.

C. Lipid handling in the intestine

The intestine is the primary site for the uptake and transport of alimentary fat. Furthermore, the intestine contributes significantly to total β-oxidation of fatty acids by the organism (152). ERRα is expressed in epithelial cells of the intestinal mucosa, and its levels of expression are particularly high in neonatal and weaning mice, a period in life when most of the energy is generated from fat (153). Gene expression profiling of the ERRα-null intestine revealed that several genes involved in OXPHOS were down-regulated, whereas β-oxidation activity in the small intestine was concurrently diminished, supporting an *in vivo* role for ERRα in energy production in that tissue (153). Another group of genes with altered expression levels in the ERRα-null intestine consisted of genes whose products participate in lipid digestion and absorption. This group included pancreatic lipase-related protein 2, fatty acid binding protein 1 and 2 (L-FABP and I-FABP), and apolipoprotein (apo)A-IV. Further characterization of the apoA-IV gene showed that ERRα directly binds to the well-studied ApoC-III enhancer, which is required for ERRα-dependent apoA-IV expression. In agreement with the gene expression data set, it was found that ERRα-null pups malabsorb dietary fats as indicated by a significant magnitude of steatorrhea. This work on lipid handling in the intestine demonstrated that ERRα is involved at another important step in the control of energy homeostasis.

D. Comprehensive orchestration of heart functions by ERRα and ERRγ

The heart has to produce a constant supply of ATP to perform its primary function as a pump. Energy demands of the heart can be met by consuming carbohydrates or fatty acids. The choice of energy substrates is governed by the developmental, physiological, and pathological status of the organism (154–157). For instance, the fetal heart predominantly uses carbohydrates as a source of energy but undergoes a postnatal shift to fatty acids to meet the increase in energy demand. In contrast, a shift from fatty acids to glucose utilization is generally observed during the pathological progression to heart failure. Functional genomics studies using a combination of ChIP-on-chip and expression profiling have shown that in cardiomyocytes, ERRα and ERRγ have the potential to regulate not only the mitochondrial programs implicated in FAO, the TCA cycle, and OXPHOS, but also gene networks involved in the uptake and processing of energy substrates, transport of ATP across membranes, generation of the phosphocreatine pool, intracellular fuel sensing, as well as Ca^{2+} handling and contraction work (26, 108). The physiological significance of these studies first became apparent when the functional and energetic response to pressure overload-induced pathological cardiac hypertrophy was compared between wild-type and ERRα-null mice (158). The expression of ERRα is diminished in the heart of wild-type mice subjected to pressure overload. In the complete absence of ERRα in the knockout mice, the heart high-energy phosphate reserves are depleted and the ATP synthesis rate is lowered, a condition that contributes to pathological remodeling in response to pressure overload. Concurrently, the expression levels of several metabolic genes identified as potential ERRα targets by ChIP-on-chip (*e.g. Acadm*, *Ckmt2*, *Etfdh*, *Hk2*, *Sdha*, *Slc25a4*) were altered in the heart of ERRα-null mice either at baseline or when subjected to pressure overload.

The expression levels of several ERR target genes included in the nuclear-encoded mitochondrial gene network (*e.g.*, *Aco2*, *Cox6a*, *Cox8a*, *Cpt2*, *Ndufs1*, *Slc25a29*) were down in the postnatal hearts of ERRγ-null mice (108). In agreement with this observation, abnormal heart function contributes to early postnatal death of the ERRγ-null mice. More specifically, the hearts of ERRγ-null mice fail to make the transition from the utilization of glucose as a fetal energy substrate to mitochondrial FAO at birth. Taken together, phenotypic analyses of ERRα- and ERRγ-null mice and associated functional genomics studies clearly demonstrated that both receptors are required to establish and maintain the cardiac bioenergetic program.

E. Mitochondrial ROS production in bone marrow-derived macrophages

The activation of macrophages by the proinflammatory cytokine IFN-γ is a major component of the defense mechanism during bacterial infection. A role for mitochondrial ROS in macrophage-mediated innate immunity had been proposed based on studies of uncoupling protein 2 (UCP-2) function (159–161). UCP-2 reduces mitochondrial ROS production by releasing membrane potential. In UCP-2-null mice, ROS levels are thus elevated, and the mice are resistant to infection by specific intracellular pathogens. Given that IFN-γ induces the expression of ERRα in bone-derived macrophages and the role of ERRs in regulating mitochondrial respiration, the interaction between IFN-γ, ERRα, and ROS production in macrophage functions was investigated using an experimental approach combining functional genomics and phenotypic analyses of knockout animals and cells (58). ChIP-on-chip studies identified several genes encoding components of the TCA cycle, electron transfer, and mitochondrial respiratory chain machinery (*e.g.*, *Aco2*, *Atp5k*, *Coq7*, *Cycs*, *Idh3a*, and *Sdhd*) as direct targets of IFN-γ-induced ERRα activity in macrophages. Consequently, ERRα-null macrophages displayed reduced mitochondrial respiratory activity, intracellular ROS levels, and inefficient clearance of pathogens in response to IFN-γ. *In vivo*, ERRα-null mice were

more susceptible to infection, a defect attributed to bone marrow-derived cells. IFN-γ-induced ERRα transcriptional activity was shown to be dependent on the presence of PGC-1β in macrophages, and the loss of PGC-1β phenocopied the loss of ERRα in mice. Therefore, ERRα acts in concert with PGC-1β to transduce the action of IFN-γ in macrophages, which results in an increase in mitochondrial ROS production and a better host innate response to bacterial pathogenesis.

V. ERRs, Energy Metabolism, and Human Diseases

Genetic evidence for a direct involvement of ERR family members in human metabolic diseases has yet to be demonstrated. However, biochemical, molecular and mouse genetic studies performed to date indicate that targeting specific ERR isoforms with pharmacological agents to modulate energy metabolism could have a beneficial impact on human diseases.

A. Obesity and diabetes

ERRα-null mice are lean and resistant to high-fat diet-induced obesity (110). As noted above, this phenotype does not agree with the primary role of ERRα in the positive regulation of energy metabolism, but highlights the more complex physiological consequence of completely restraining ERRα activity in the whole organism. For example, UCP-1 expression is up-regulated in ERRα-null WAT by a mechanism that has yet to be characterized, a change in WAT function that may contribute to an increase in energy expenditure and lower body weight. Because ERRα has obvious ligand-independent activities, it is not clear whether the utilization of a potent ERRα inverse agonist would result in a similar phenotype. A potential link between the polymorphic sequence that alters the copy number of a functional ERRE in the promoter of the ERRα gene in human obesity was also investigated. One group observed that a higher ERRE copy number was associated with a higher body mass index in Japanese individuals (162). This result would agree with the ERRα-null mice phenotype because higher expression of ERRα would lead to a higher body weight. However, no such genetic linkage was found in a larger population-based study of Danish Caucasians (163).

Mitochondrial dysfunctions have been linked to type 2 diabetes (164). In particular, defects in mitochondrial FAO may contribute to insulin resistance in humans via an increase in intracellular fatty acid metabolites affecting insulin signaling. Indeed, the expression of a subset of genes involved in OXPHOS was shown to be down-regulated in human diabetic muscle (56). This subset of genes can be up-regulated by the introduction of PGC-1α in C2C12 myotubes in a manner that is dependent on the presence of ERRα (90). Because ERRα has an opposite effect on the expression of genes involved in gluconeogenesis (114), tissue-selective modifiers of ERRα activity could constitute valuable pharmacological tools to increase mitochondrial function and FAO in muscle while at the same time lowering gluconeogenesis in liver and plasma glucose levels.

B. Heart failure

The development and progression of chronic heart failure is multifactorial, but changes in bioenergetics are an important component of the disease (157). As described above, recent studies have shown that ERRα and ERRγ regulate entire metabolic programs in both the developing and adult heart and that abolishing ERRα and ERRγ function has dramatic physiological consequences for the heart (26, 108, 158). In particular, the presence of ERRα is essential for the bioenergetic adaptation of the heart subjected to hemodynamic stressors known to cause heart failure. Moreover, a large number of ERRα and ERRγ target genes involved in other aspects of heart functions (*e.g.*, contractile, structural) have been linked to cardiac phenotypes in mouse models and/or cardiomyopathies in humans (26, 108). It is thus conceivable that, by altering cardiac energy metabolism and other functions of the heart, ERR ligands could provide new therapeutic avenues for the prevention and treatment of heart failure.

C. Cancer cell metabolism

One of the hallmarks of cancer cells is their acquired dependency on glycolysis for energy production in the context of adequate oxygen to sustain glucose oxidation. This physiological alteration in energy metabolism, *e.g.*, paradoxical aerobic glycolysis, is referred to as the Warburg effect and is now believed to be required for malignant progression (165, 166). The ERRs promote mitochondrial respiration, and a reduction in their activity could support a shift toward glycolysis. However, this attractive hypothesis remains to be tested. In contrast, a large-scale proteomic analysis of a human breast cancer model for brain metastasis has shown that ERRα, PGC-1α, and PGC-1β, as well as proteins involved in FAO and OXPHOS are up-regulated in these cells (167). The switch of the parent cell line from anaerobic glycolysis toward increased mitochondrial metabolism of glucose in the brain-derived cells may be a requirement to adapt to the energy pathways predominant in the brain and contribute to the growth advantage of tumor cells in the brain microenvironment.

ERRs have also been shown to be possibly involved in the transcriptional response to hypoxia and growth of solid tumors. The adaptive response to low oxygen levels is mainly controlled by a transcription factor referred to as hypoxia-inducible factor (HIF). HIF regulates gene networks involved in glucose uptake and metabolism as well as tumor angiogenesis. ERRs can directly interact with HIF *in vitro* and *in vivo* and enhance HIF-mediated gene transcription, whereas inhibition of ERR activity attenuates the hypoxic response (168).

Modulation of ERR activity could also potentially be used to control cachexia in cancer patients. Cachexia, a chronic hypermetabolic state stimulated by high levels of inflammatory cytokines, is observed in more than 50% of cancer patients. It has been shown that cytokines activate PGC-1α via phosphorylation by p38 MAPK, resulting in an increase in the expression of genes associated with mitochondrial uncoupling and energy expenditure (169). Given that ERRα has been shown to modulate cytokine activation of mitochon-

drial functions via PGC-1β in macrophages (58), inhibiting ERR action in muscle could be beneficial to cachexic cancer patients.

VI. Closing Remarks

Although the ERRs were identified two decades ago, the realization that these receptors play crucial roles in controlling energy homeostasis is only beginning to emerge. Indeed, a recent in-depth review on the transcriptional control of metabolism made no mention of the ERRs (1). In contrast, the studies reviewed herein have clearly established that the ERRs regulate vast gene networks involved in a wide range of bioenergetic processes (Fig. 5). In particular, the ERRs can now be considered as essential factors for normal mitochondrial biogenesis and function. Loss of a single ERR isoform can lead to mitochondrial dysfunctions and related physiological consequences such as cold intolerance, depletion of energy stores in the heart, and diminished ROS production in macrophages with associated susceptibility to bacterial infection. Mechanistically, the ERRs appear to be the major conduits for the action of PGC-1α and related isoforms in the control of energy metabolism. However, it is currently unknown whether the sole presence of one of the PGC-1 isoforms is sufficient to activate the ERRs, or whether the interactions between the ERRs and the PGC-1 coactivators can be modulated by signals specifically targeting the receptors. These signals could take the form of one or more endogenous ligands or posttranslational modification of the receptors induced by extracellular events. The same principle applies for the interaction between the ERRs and the corepressor RIP140 and how the exchanges between coactivators and corepressors are modulated. Identification of these signals and establishing their physiological relevance in health and diseases are the foremost challenges facing investigators in this field.

Although molecular studies have indicated that the three ERR isoforms share a considerable amount of structural and functional features, analyses of genetically modified mice have shown that the three ERRs display stricter specificity in their primary biological functions. Nonetheless, genetic redundancy between receptor isoforms was also evident, as exemplified by the fact that both the ERRα- and ERRβ-null mice are viable under certain experimental conditions, and that ERRα and ERRγ can function as heterodimeric partners and control identical gene networks. Tissue-specific loss or gain of function approaches will be required to uncover the exact roles played by each receptor isoform in distinct metabolic organs.

Given their extended roles in the transcriptional control of energy homeostasis, the ERRs represent leading targets for therapeutic interventions of metabolic diseases such as obesity, diabetes, and heart failure, as well as cancer. Potent modulation of ERR activity by pharmacological agents has proven elusive so far, a feature that may be related to the constitutive interactions observed between the ERRs and their coregulators. An alternative strategy would be to modulate the activity of ERR coactivators as recently shown for resveratrol-induced PGC-1α activity via SIRT1 activation (170). Despite these hurdles, there is good reason to be hopeful that the ERRs will soon join the long list of nuclear receptors successfully targeted for therapeutic intervention.

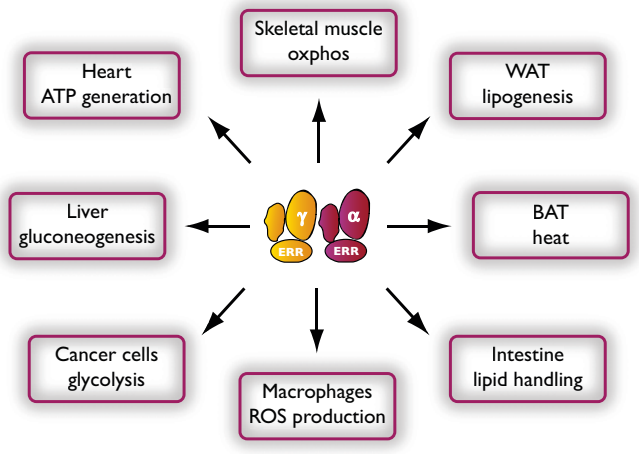

FIG. 5. Tissue-specific functions related to the control of energy metabolism by ERRα and/or ERRγ.

Acknowledgments

The author thanks Catherine R. Dufour for production of Table 1 and members of the laboratory for suggestions.

Received May 2, 2008. Accepted July 21, 2008.

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This work was supported by the Canadian Institutes for Health Research, the National Cancer Institute of Canada via a Terry Fox Program Project Grant and the Fonds de la recherche en sante du Quebec.

Disclosure Statement: The author has nothing to declare.

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