The metabolic coregulator RIP140: an update

Asmaa Fritah, Mark Christian, and Malcolm G. Parker

Institute of Reproductive and Developmental Biology, Imperial College London, Faculty of Medicine, Du Cane Road, London, United Kingdom

Submitted 22 April 2010; accepted in final form 3 June 2010

Fritah A, Christian M, Parker MG. The metabolic coregulator RIP140: an update. Am J Physiol Endocrinol Metab 299:E335–E340, 2010. First published June 8, 2010; doi:10.1152/ajpendo.00243.2010.—RIP140 is a transcriptional coregulator that controls energy homeostasis and metabolism through direct regulation of glucose transport in adipocytes. In this review, we focus on recent advances highlighting the growing importance of RIP140 as a regulator of energy homeostasis.

TRANSCRIPTIONAL COREGULATORS provide an important level of control in a number of metabolic processes. The importance of activating gene expression in metabolic pathways controlling energy homeostasis is well established, whereas the contribution of transcriptional inhibition is less well defined. Recent evidence indicates that receptor-interacting protein-140 (RIP140) is a multifunctional coregulator with a central role in metabolic tissues (19). RIP140 was originally identified by its ability to interact with estrogen receptors and found to repress their transcriptional activity (5). Subsequently, RIP140 was found to bind and repress a number of other nuclear receptors, including the peroxisome proliferator-activated receptors (PPARα, PPARβ/δ, and PPARγ), thyroid hormone receptors (TRα and TRβ), and estrogen-related receptors (ERRα and ERRβ), particularly important in regulating gene expression in metabolic tissues, specifically adipose tissue, muscle, and liver (8).

The biological role of RIP140 as a metabolic coregulator became clear through the analysis of a loss-of-function mouse model. RIP140-null mice are extremely lean and exhibit resistance to obesity and hepatic steatosis as well as enhanced glucose tolerance and enhanced responsiveness to insulin compared with matched wild-type littermates fed a high-fat diet (19). In these mice, expression profiling identified the key metabolic genes uncoupling protein-1 (UCP1) (19), cell death-inducing DFF45-like effector A (6), and glucose transporter 4 (GLUT4) (25) as targets for repression in adipocytes, whereas fatty acid-binding protein-3 and medium-chain acyl-CoA dehydrogenase expressions were suppressed in skeletal muscle (29). Depletion of RIP140 in 3T3-L1 adipocytes as well as experiments performed in RIP140-null myotubes confirmed that RIP140 acts as a transcriptional corepressor modulating expression of genes implicated in glucose uptake, glycolysis, tricarboxylic acid cycle, fatty acid oxidation, mitochondrial biogenesis, and oxidative phosphorylation (6, 9, 14, 25). As a consequence, in the absence of RIP140, white adipose tissue (WAT) acquires a number of features reminiscent of brown adipose tissue (BAT), notably the upregulation of UCP1 (19) (Fig. 1). The suppression of UCP1 gene expression and potentially other catabolic genes in WAT seems to depend on the recruitment of chromatin remodeling enzymes. Analysis of the Ucp1 promoter and upstream enhancer suggests that RIP140 functions as a scaffold for both DNA and histone methyltransferases to inhibit gene transcription by two key epigenetic repression systems (18) (Fig. 2).

Paradoxically, it has emerged that RIP140 is also capable of activating transcription. For example, RIP140 was shown to activate inflammatory gene expression in macrophages (37), triglyceride synthesis in the liver (14), and, more recently, amphiregulin expression in the ovary to promote ovulation (23). In this review, we focus on recent advances in our understanding of the function of RIP140 as a regulator of energy homeostasis.

RIP140: Corepressor vs. Coactivator

There is substantial evidence to indicate that RIP140 functions as both a corepressor in adipose and muscle to control the state of adiposity (19), muscle fibre types and metabolism (9, 29)
and glucose tolerance (25) (Fig. 1). In the liver, we have found that RIP140 not only acts as a corepressor but is also capable of acting as a coactivator (14). Liver X receptor (LXR) is a nuclear receptor that plays a key role in regulating lipogenesis in the liver through modulating the expression of another transcription factor, namely sterol regulatory binding-protein-1c (SREBP-1c) and the enzyme fatty acid synthase (FAS). RT-PCR performed on primary hepatocytes isolated from RIP140-null mice showed that RIP140 acts as a corepressor for LXR in controlling the expression of PEPCK and, in the same cells, acts as coactivator for LXR-dependent regulation of SREBP-1c and FAS (14). Similarly, depletion of RIP140 in HuH7 cells by infection with an adenovirus encoding RIP140 shRNA recapitulates the alterations in gene expression observed in the liver of RIP140-null mice, suggesting that the action of RIP140 is cell autonomous. In support of RIP140 as a coactivator, we have shown that it upregulates FAS promoter activity in a dose-dependent manner. Furthermore, chromatin immunoprecipitation experiments performed on HuH7 chromatin extracts show that RIP140 and LXR are simultaneously recruited to the FAS promoter region containing the LXR-responsive element. In accord with those results, RIP140-null mice fail to develop steatosis when fed a high-fat diet (19) (Fig. 1) which points to RIP140 as a coactivator for LXR in controlling FAS expression in the liver. Another study found a corepressor role for RIP140 in LXR-dependent expression of
FAS and SREBP-1c in liver cells (2), but we are unable to provide an explanation for the apparent discrepancy. Other studies demonstrate that RIP140 behaves as a coactivator for a number of other transcription factors in a variety of cellular responses (23, 37). In macrophages, RIP140 promotes the activity of NF-κB and upregulates the expression of genes implicated in inflammation such as TNFα and interleukin-6 (37). The mechanism by which RIP140 acts as a coactivator is through direct interaction with NF-κB subunit Rel A and the transcriptional coactivator CREB-binding protein (CBP) via the NH2-terminal domain of RIP140 (Fig. 2). More recently, we have shown that RIP140 acts as a coactivator for CREB/c-jun family members in upregulating the expression of amphiregulin in granulosa cells (23, 32). In RIP140-null mice, a deficiency in amphiregulin expression leads to a failure in cumulus expansion and a failure to ovulate, which is partly responsible for female infertility (19, 36). All together, these studies highlight a new function for RIP140 as a transcriptional coactivator. The dual function of RIP140 as a coactivator or corepressor implies a switch in the assembly of chromatin remodeling enzymes that might depend on protein-protein interactions determined by promoter-specific elements and/or transcription factors (Fig. 2) or posttranslational modifications brought about by other signaling pathways (see below).

Although the consequences of RIP140 depletion have been extensively studied, the effects of overexpression have only recently been characterized in mice expressing exogenous RIP140. The most obvious phenotype of mice expressing exogenous RIP140 is impaired postnatal heart function (10). The transgenic mice are characterized by rapid onset of cardiac hypertrophy and ventricular fibrosis (Fig. 1) resulting in increased mortality from 4 wk of age. Interestingly, females are less sensitive to the deleterious effects of exogenous RIP140 expression than males, suggesting that estrogens might play a protective role against cardiac hypertrophy as supported by other studies (34). In these mice, overexpression of RIP140 leads to reduced expression of genes implicated in fatty acid transport/oxidation and mitochondrial activity. This is accompanied by a decrease in mitochondrial number and activity, as demonstrated by decreased state III and state IV membrane potential and oxygen consumption, associated with abnormal morphology (Fig. 1). Thus, decreased energy production associated with increased fibrosis might be responsible for impaired cardiac function and decreased survival observed in the RIP140 transgenic mice. This study is consistent with data for skeletal muscle and myotubes obtained from RIP140-null mice, where absence of RIP140 leads to increased oxidative metabolism (9, 14) (Fig. 1). Thus, it highlights the importance

---

**Fig. 2. Dual coregulator function of RIP140 in regulating target gene transcription.**

**A** RIP140 acts as a transcriptional corepressor for PPARs, blocking UCP1 promoter activation through the recruitment of DNA methyltransferase (Dmnt), COOH-terminal binding protein (CtBP), histone methyltransferase (HMT), and histone deacetylase (HDAC).

**B** RIP140 acts as transcriptional coactivator for NF-κB to activate promoters of IL-6 and -1β (IL1β) through recruitment of histone acetyltransferase CREB-binding protein (CBP).
of RIP140 in postnatal cardiac function and reinforces the role of RIP140 as a metabolic corepressor. Moreover, it is apparent that the RIP140 transgenic mice are not obese, but it should be noted that 1) the ratio of exogenous to endogenous RIP140 expression is less in adipose tissue than in cardiac muscle, and 2) many of the mice die prematurely from heart failure and so the progressive increase in fat accumulation noted in wild-type mice does not have time to develop.

**Regulation of RIP140 Expression**

Analysis of RNA from a number of mouse and human cell lines and tissues indicates that the RIP140 gene comprises multiple well-conserved noncoding exons with alternative splicing to a single coding exon (1, 24). The RIP140 promoter region extends to more than 100 kilobases upstream of the coding exon, and alternate transcription start sites, together with differential splicing, lead to a complex repertoire of RIP140 mRNAs with distinct untranslated regions but encoding the same protein. The relative amounts of alternative transcripts vary in different cell types, suggesting that their expression may be subject to differential regulation. Regarding the role of RIP140 as an important modulator of nuclear receptor transcriptional activity, studies have shown that RIP140 expression is regulated by estrogens (1), retinoic acid (17), progestins (11), and androgens (3). This is likely to be an important mechanism to prevent excessive nuclear receptor target gene expression and has been identified in MCF-7 breast cancer cells, in which RIP140 is an early estrogen-induced gene that is required for late gene repression (4). ERRs, implicated in the control of energy metabolism, also modulate RIP140 transcription (24). Recently, it has been demonstrated that microRNAs regulate the expression of RIP140 (31). A novel splice variant of RIP140 has been discovered, specifically expressed in the brain, that is targeted in the 5' UTR by mir-346 (31). The action of RIP140 in the brain is not clearly established, and this study highlights the need to study it further. Expression of mir-346 leads to increased RIP140 protein accompanied by increased RIP140 corepressive activity without affecting the RIP140 mRNA level. Mir-346 is generated from the gene encoding glutamate receptor ionotropic delta 1 (GRID1) in the brain and has been implicated in schizophrenia and inflammation. A recent study has established a role for RIP140 as a proinflammatory factor (37), and it is well known that inflammation is implicated in the development of insulin resistance. Thus, it would be of interest to search for other sites of expression for mir-346, particularly adipose tissue, skeletal muscle, and macrophages and ascertain its involvement in the regulation of RIP140 function. The discovery of new microRNAs targeting RIP140 should also be investigated further.

**Regulation of RIP140 Activity**

There is evidence that RIP140 is subject to posttranslational modifications (7); however, little is known about the consequences of such modifications on RIP140 function. It is particularly important to decipher whether the action of RIP140 either as a coactivator or as a corepressor can be regulated by environmental stimuli such as fasting, exercise, or high-fat diet, leading to activation of specific signaling pathways. RIP140 is subject to ubiquitination, sumoylation (28), acetylation, phosphorylation, and methylation, and a number of these modifications have been shown to affect its function (22). RIP140 acts as a scaffold protein for the recruitment of additional factors such as histone deacetylases (35), DNA and histone methyltransferases (18), and COOH-terminal binding protein (CtBP), which mediate the corepressive function of RIP140 (33) (Fig. 2). CtBP association can be abolished by the acetylation of RIP140 by CBP (33). Conversely, RIP140 acts as a coactivator when bound to CBP (37). Thus, exchange of cofactors bound to RIP140 in transcriptional regulating complexes could be influenced by posttranslational modifications affecting RIP140. Increasing our knowledge about the kinetics of events at promoter sites will help us understand better how RIP140 function is regulated and how in turn it influences gene expression.

The way RIP140 modulates gene expression has been extensively studied during the past decade. We have established that it acts as a transcriptional corepressor for nuclear receptors controlling energy homeostasis in the adipose tissue and skeletal muscle. On the other hand, PPARα coactivator-1α (PGC-1α) has emerged as the main metabolic tissue coactivator, which stimulates adaptive thermogenesis and mitochondrial biogenesis (26). Remarkably, most of the genes repressed by RIP140 are activated by PGC-1α (6, 14). Recently, we have shown that RIP140 interacts directly with PGC-1α and suppresses its activity (13), strengthening the mutually antagonistic...
tic functions of the two coregulators. The direct antagonism of PGC-1α by RIP140 may provide the basis of a rheostat that could determine the extent to which metabolic target genes are expressed. In addition to the role of RIP140 in the function of white adipocytes, it is conceivable that it also plays a role in the development of BAT, since newborn RIP140-null mice have reduced BAT mass and exhibit a high death rate (16). Nevertheless, the ability of BAT to respond to β3-adrenergic stimulation is maintained in RIP140-null mice.

Although RIP140 is predominantly a nuclear protein, its localization seems to be regulated by alternative signaling pathways. RIP140 is subject to sumoylation, and this is accompanied by a relocalization of RIP140 from small nuclear foci (30) to a more diffuse nuclear distribution (28). This correlates with an increase in RIP140 transcriptional repressive activity. Thus, the intranuclear localization of RIP140 is tightly regulated, suggesting that sequestration of RIP140 into small nuclear loci might help in activating gene transcription. Furthermore, coexpression of RIP140 alters the nuclear distribution of PGC-1α, which adopts a granular nuclear distribution by colocalizing with RIP140 (27). The functional interaction between these coregulators is modulated by sumoylation of PGC-1α (27). On the other hand, RIP140 methylation by protein arginine N-methyltransferase-1 (PRMT1) leads to RIP140 nuclear export and inhibition of its trans-repressive properties (21), suggesting that RIP140 relocalization into the cytoplasm blocks its capability to act as a transcriptional corepressor. Interestingly, nuclear export of RIP140 has been shown to be triggered by its phosphorylation by PKCe followed by arginine methylation in adipocytes (12). When phosphorylation-deficient RIP140 was reintroduced into RIP140-null adipocytes, the defect in fat accumulation was rescued (12). Thus, cytoplasmic localization of RIP140 can be seen as a supplementary mechanism to move RIP140 away from target promoters and enables the expression of genes implicated in fatty acid oxidation.

A role for RIP140 in the cytoplasm has been identified by Wei et al. (15), distinct from that in transcriptional regulation. Cytoplasmic RIP140 inhibits glucose metabolism by reducing insulin-stimulated GLUT4 trafficking and glucose uptake (15). This is the consequence of RIP140 interacting with AS160 and impeding its phosphorylation/inactivation by Akt (Fig. 3). Importantly, the same study shows that high-fat feeding results in cytoplasmic localization of RIP140 in epididymal white adipocytes, strengthening the biological relevance of a function for RIP140 in the cytoplasm. This provides the basis for a novel mechanism by which RIP140 impairs glucose utilization and promotes insulin resistance. Importantly, the cytoplasmic role of RIP140 is in addition to the direct regulation of GLUT4 mRNA expression by RIP140 in mouse and human adipocytes (20, 25).

Final Words

Recent advances have shown the growing importance of RIP140 in regulating fatty acid and glucose metabolism in adipose tissue, skeletal muscle, the heart, and the liver. Although displaying pleiotropic effects, RIP140 acts in the nucleus as well as in the cytoplasm as a transcriptional corepressor as well as a coactivator to ultimately repress energy utilization. As a result of the ability of RIP140 to regulate many different signaling pathways in the liver, adipose tissue, and skeletal muscle, targeting RIP140 by specific or a combination of treatments may participate in preventing or delaying the onset of cardiac dysfunction as well as the development of obesity and diabetes. To avoid the deleterious effect observed in the reproductive tract of RIP140-null females, any drug designed to target metabolic action of RIP140 should be tissue specific. Thus, increasing our knowledge about potential tissue-specific posttranslational modifications as well as transcriptional regulation of RIP140 might help in dealing with such issues.

DISCLOSURES

No conflicts of interest are reported by the authors.

REFERENCES


