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Targeting Sirtuin 1 to Improve Metabolism: All You Need Is NAD$^+$?

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***Abstract*——Sirtuin 1 (SIRT1) is an evolutionarily conserved NAD$^+$-dependent deacetylase that is at the pinnacle of metabolic control, all the way from yeast to humans. SIRT1 senses changes in intracellular NAD$^+$ levels, which reflect energy level, and uses this information to adapt the cellular energy output such that it matches cellular energy requirements. The changes induced by SIRT1 activation are generally (but not exclusively) transcriptional in nature and are related to an increase in mitochondrial metabolism and antioxidant protection. These attractive features have validated SIRT1 as a therapeutic target in the management of metabolic disease and prompted an intensive search to identify pharmacological SIRT1 activators. In this review, we first give an overview of the SIRT1 biology with a particular focus on its role in metabolic control. We then analyze the pros and cons of the current strategies used to activate SIRT1 and explore the emerging evidence indicating that modulation of NAD$^+$ levels could provide an effective way to achieve such goals.**

I. Introduction

During the last decade the mammalian sirtuin (SIRT[1]) family (formed by paralogs SIRT1–SIRT7) has emerged as

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[1]Abbreviations: AceCS, acetyl-CoA synthetase; AMPK, AMP-activated protein kinase; AROS, active regulator of SIRT1; ChREBP, carbohydrate response element-binding protein; CREB, cAMP response element-binding protein; CRTC, CREB-regulated transcriptional coactivator; CtBP, C-terminal binding protein; DBC, deleted in breast cancer; eNOS, endothelial nitric-oxide synthase; ERC, extrachromosomal rDNA circle; FOXO, Forkhead-O-box; HIC1, hypermethylated in cancer 1; JNK, c-Jun NH_2-terminal kinase; LXR, liver X receptor; miRNA, microRNA; NA, nicotinic acid; NAM, nicotinamide; Nampt, nicotinamide phosphoribosyltransferase; NCoR, nuclear receptor corepressor; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; PARP, poly(ADP-ribose) polymerase; PGC, PPARγ coactivator; PPAR, peroxisome proliferator-activated recep-

a constellation of enzymes with key roles in whole-body metabolic homeostasis and an interesting therapeutic potential applicable to multiple pathophysiological states.

The history of sirtuins began almost 3 decades ago, with the identification of Sir2 (silent information regulator 2), a protein-forming part of a complex that enabled gene silencing at selected regions of the yeast genome (Shore et al., 1984; Ivy et al., 1986). A major turning point in the history of Sir2 came from the discovery that Sir2 was involved in the yeast replicative aging process (Kaeberlein et al., 1999). The accumulation of extrachromosomal rDNA circles (ERCs) as the organism ages is believed to be a major determinant of yeast replicative lifespan (Sinclair and Guarente, 1997). Although the mechanism by which the accumulation of ERCs influences lifespan is not fully understood, different genetic manipulations promote reasonable, despite correlational, evidence that the accumulation of ERCs is negatively correlated with yeast replicative aging (Sinclair and Guarente, 1997; Defossez et al., 1999; Kaeberlein et al., 1999). It was originally thought that the impact of Sir2 on replicative lifespan of yeast was consequent to its silencing activity on ERCs. However, the effects of Sir2 on aging extend further than ERC silencing, because genetic manipulations of Sir2 orthologs can also affect lifespan of higher eukaryotes, such as the nematode *Caenorhabditis elegans* (Tissenbaum and Guarente, 2001; Viswanathan et al., 2005; Berdichevsky et al., 2006; Rizki et al., 2011) and insects such as *Drosophila melanogaster* (Rogina and Helfand, 2004; Bauer et al., 2009), where ERCs are not thought to cause aging. However, there are some caveats on the consistency, amplitude, and mammalian translation of the lifespan-extension effects of Sir2 orthologs (Kaeberlein and Powers, 2007; Burnett et al., 2011; Lombard et al., 2011; Viswanathan and Guarente, 2011), which suggest that the effects of Sir2 on organismal lifespan might be indirect and/or largely depend on a specific repertoire of third-party modulators.

If not acting primarily as lifespan determinants, what then is the exact function of this family of proteins? A first hint of the real function of Sir2, or its orthologs, was grasped when the activity of Sir2 as a silencing enzyme was more precisely defined as a NAD$^+$-dependent deacetylase (Imai et al., 2000). In the reaction catalyzed by sirtuins, an acetylated substrate gets deacetylated, using NAD$^+$ as a cosubstrate, and yielding the deacetylated substrate, nicotinamide, and 2′-*O*-acetyl-ADP-ribose (Fig. 1). The NAD$^+$ dependence and the relatively high K_m of the Sir2 enzyme for NAD$^+$ immediately suggested a potential link between Sir2 activity and the metabolic state of the cell (Guarente, 2000).

In mammals, there are seven Sir2 orthologs (SIRT1–SIRT7) that constitute the sirtuin family of enzymes. All of them are ubiquitously expressed and share a conserved catalytic core comprising 275 amino acids (for review, see Dali-Youcef et al., 2007; Michan and Sinclair, 2007). The different members of the mammalian sirtuin family, however, show distinct features that probably endow them with specialized functions. For example, mammalian sirtuins differ in their subcellular localization. SIRT1, the best characterized family member, resides mainly in the

tor; PPRE, PPAR-response element; Sir, silent information regulator; SIRT, sirtuin; SREBP, sterol regulatory element binding protein; SRT1720, *N*-[2-[3-(piperazin-1-ylmethyl)imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]quinoxaline-2-carboxamide; SUMO, small ubiquitin-like modifier; UCP, uncoupling protein.

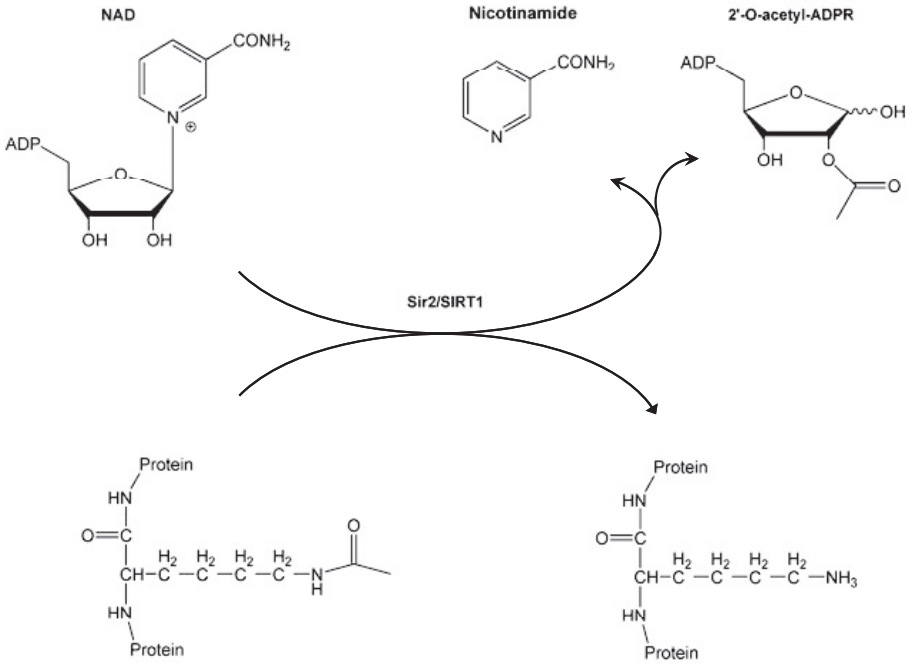


FIG. 1. The NAD$^+$-dependent SIRT1 deacetylase reaction. SIRT1 uses NAD$^+$ as a substrate to remove acetyl groups from a target protein. In addition to the deacetylated substrate, the reaction yields nicotinamide and 2′-*O*-acetyl-ADP ribose (2′-*O*-acetyl-ADPR) as products.

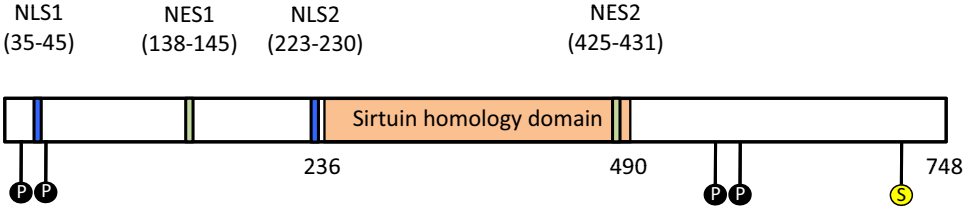
nucleus (Michishita et al., 2005) but can shuttle from the nucleus to the cytosol (Tanno et al., 2007), where several of its targets are found. SIRT2 is localized mainly in the cytoplasm, although it can also regulate gene expression by deacetylation of transcription factors that shuttle from the cytoplasm to the nucleus (Jing et al., 2007), and it contributes to chromatin compaction upon disassembly of the cell nucleus during mitosis (Vaquero et al., 2006). SIRT3, SIRT4, and SIRT5 are generally considered mitochondrial proteins (Onyango et al., 2002; Schwer et al., 2002; Michishita et al., 2005), whereas SIRT6 and SIRT7 are nuclear proteins. However, although SIRT6 is located predominantly in the heterochromatin, SIRT7 is thought to be mainly enriched in the nucleoli (Michishita et al., 2005).

In addition to their differential cellular locations, the sirtuin family members can also be distinguished by their different enzymatic activities. SIRT1 and SIRT5 act as deacetylases (Imai et al., 2000; Vaziri et al., 2001), whereas SIRT4 seems to be a mono-ADP-ribosyl transferase (Haigis et al., 2006). SIRT2, SIRT3, and SIRT6 can display both activities (North et al., 2003; Liszt et al., 2005; Shi et al., 2005; Michishita et al., 2008). The activity of SIRT7 has not been clearly established, even though it has been hypothesized to act as a deacetylase (Vakhrusheva et al., 2008). It is noteworthy that SIRT5 was recently described to demalonylate and desuccinylate proteins (Du et al., 2011; Peng et al., 2011). It is tempting to speculate that the spectrum of action of sirtuin is not limited to deacetylation but would cover a much wider range of acylation-based post-translational modifications. The identification of sirtuin substrates during the last few decades has clearly pointed out a prominent role of sirtuins as metabolic regulators. For the purpose of this review, we mostly focus on the actions of SIRT1. For extensive discussion of the actions of other sirtuin members, we refer the reader to reviews elsewhere (Dali-Youcef et al., 2007; Michan and Sinclair, 2007; Yamamoto et al., 2007; Schwer and Verdin, 2008; Finkel et al., 2009; Guarente, 2011).

II. Sirtuin 1 in a Nutshell

A. Sirtuin 1: What and Where Is It?

Among all sirtuins, SIRT1 is the best characterized. Human SIRT1 contains the conserved catalytic core of sirtuins and both N- and C-terminal extensions that all span ~240 amino acids (Fig. 2). These extensions serve as platforms for interaction with regulatory proteins and substrates. In total, the human SIRT1 spans 747 amino acids. SIRT1 contains two nuclear localization signals as well as two nuclear exportation signals (Tanno et al., 2007). The balanced functionality of these signals determines the presence of SIRT1 in either the nuclear or the cytoplasmic compartment and explains why SIRT1 location may differ depending on the cell type or tissue evaluated. For instance, although SIRT1 is found mainly in the nuclear compartment in COS-7 cells (McBurney et al., 2003; Sakamoto et al., 2004), it is abundantly found in the cytosol of



	Region / aminoacids	Function	Reference
Sirtuin homology domain	aa 244-498	enzymatic acitivity	Frye, 1999
Nuclear Localization Signal	NLS1 (aas 34-44) NLS2 (aas 232-239)	Nuclear importation	Tanno et al., 2007
Nuclear Exportation Signal	NES1 (aas 146-?) NES2 (aas 435-443)	Nuclear exportation	Tanno et al., 2007
Phosphorylation	(by JNK1) Ser27, Ser47, Thr530	Specific activation towards H3, but not p53	Nasrin et al., 2009
	(by Cyclin/cdk1) Thr530, Ser540	Increases deacetylase activity	Sasaki et al., 2008
Sumoylation	Lys734	Increases deacetylase activity	Yang et al., 2007c

FIG. 2. Relevant domains in the human form of the SIRT1 protein. The figure schematizes the span of the conserved sirtuin homology domain as well as the nuclear localization signal (NLS) and nuclear exportation signals (NES). The residues subject to phosphorylation by JNK1 and Cyclin/cdk1 and by SUMOylation are also indicated.

rodent β cells, myotubes, and cardiomyocytes (Moynihan et al., 2005; Tanno et al., 2007). Although the implications and regulation of SIRT1 shuttling are still largely unknown, some experiments indicate that SIRT1 shuttles from the nuclei to the cytosol upon inhibition of insulin signaling (Tanno et al., 2007). The latter observations suggested a link between SIRT1 activity and the sensing of the metabolic status of the cell, as discussed in the next chapter.

B. Sirtuin 1 as an NAD$^+$ Sensor

SIRT1 activity is generally increased in situations of energy/nutrient stress. The fact that SIRT1 activity is regulated by NAD$^+$ raised the hypothesis that NAD$^+$ could act as a metabolic sensor in situations of energy stress, where NAD$^+$ levels are generally affected. Some aspects of this hypothesis are still controversial (for review, see Cantó and Auwerx, 2009). First among them is whether sirtuin activity can really respond to changes in intracellular NAD$^+$ levels. One premise, at least, must be met in that context: that the K_m of the sirtuins for NAD$^+$ falls into the physiological range of NAD$^+$ bioavailability. Direct experimental evidence supporting this point is, in most cases, preliminary or absent. In great part, this is because the true bioavailable NAD$^+$ levels remain difficult to evaluate. The estimated total intracellular content of NAD$^+$ is in the 0.2 to 0.5 mM range (for review, see (Sauve et al., 2006; Houtkooper et al., 2010), which lies within the estimated K_m values of SIRT1 for NAD$^+$. This would indicate that NAD$^+$ might actually be rate-limiting in certain circumstances to propel SIRT1 to its maximal activity. These levels, however, do not differentiate free and protein-bound NAD$^+$. Likewise, this approximation does not take into account the existence of cellular compartmentalization of NAD$^+$.

Changes in intracellular NAD$^+$ rarely fluctuate more than 2-fold (Rodgers et al., 2005; Chen et al., 2008; Fulco et al., 2008; Cantó et al., 2009), which is a likely range to affect sirtuin activity. In general, NAD$^+$ levels increase in mammalian tissues in response to energy/nutrient stresses such as exercise (Cantó et al., 2009, 2010; Costford et al., 2010), fasting (Rodgers et al., 2005; Cantó et al., 2010), or calorie restriction (Chen et al., 2008). Accordingly, SIRT1 activity is enhanced by all these conditions. It is noteworthy that NAD$^+$ levels have been reported to fluctuate in a circadian fashion (Nakahata et al., 2009; Ramsey et al., 2009). The influence of SIRT1 on the control of clock-related gene expression (Asher et al., 2008; Nakahata et al., 2008) makes it very attractive to conceive this relation as a way by which feeding/fasting cycles influence the circadian clock. In general, high glycolytic rates in the fed state would bring about higher NAD$^+$ reduction rates, whereas the reduced glycolytic rate in the fasted state would enhance mitochondrial oxidative metabolism, derived from fatty acid oxidation, which is generally paired with higher NAD$^+$ levels. This scenario constitutes a beautiful mechanism by which metabolism would be directly coupled to the enzymatic activity of SIRT1 and downstream pathways.

SIRT1 activity is also controlled by other NAD$^+$-derived metabolites. It was proposed that NADH would compete with NAD$^+$ binding to SIRT1 and inhibit SIRT1 activity (Lin et al., 2004). However, NADH can competitively inhibit NAD$^+$ binding only in the millimolar range, which is well above its physiological levels (Schmidt et al., 2004). A more prominent and consistent inhibitory effect is achieved with nicotinamide (NAM), which exerts a potent end-product inhibition on SIRT1 activity in a noncompetitive fashion with NAD$^+$ (Bitterman et al., 2002; Anderson et al., 2003). Kinetic studies demonstrate that NAM acts at a K_m between 30 and 200 μM (Bitterman et al., 2002). The reference values for the intracellular concentration and subcellular compartmentalization of NAM are still far from determined, an issue that is further complicated by the diffusive nature of NAM (van Roermund et al., 1995). Indirect evidence of the large influence of NAM on SIRT1 activity is derived from experiments that manipulate NAM metabolism through changing the activity of nicotinamide phosphorybosyltransferase (Nampt). In the cell, NAM is used as a substrate for NAD$^+$ resynthesis through the action of Nampt (Revollo et al., 2004). Inhibition or downregulation of Nampt leads to NAM accumulation and NAD$^+$ depletion, ultimately decreasing SIRT1 activity (Revollo et al., 2004). This highlights how NAM can influence SIRT1 activity through different means: first, as a noncompetitive SIRT1 inhibitor, and, second, as an NAD$^+$ precursor. Low levels of NAM might therefore be beneficial for SIRT1 activity, because NAM can act as an NAD$^+$ precursor, but, more importantly, accumulation of NAM could be deleterious through the inhibition of SIRT1 (Yang and Sauve, 2006).

C. Sirtuin 1 Actions

1. Nuclear Targets. In agreement with its dual cellular localization, SIRT1 targets can be found in both the nuclear and cytosolic compartments. SIRT1 activity in the nucleus articulates dynamic and varied transcriptional responses through the deacetylation of a large spectrum of transcriptional regulators. Therefore, the deacetylation by SIRT1 can lead to the direct activation or inhibition of transcriptional regulators and modify their interaction profiles, depending on the cellular context. From a metabolic perspective, it is exciting to see that many SIRT1 deacetylation targets are key metabolic regulators, further enhancing the notion that SIRT1 is a metabolic stress effector governing transcriptional adaptations aimed to synchronize energy metabolism with nutrient availability. SIRT1 activity and targets, however, expand beyond the realm of metabolism. For example, SIRT1 has marked anti-inflammatory effects in diverse tissues and cell models (Pfluger et al., 2008; Purushotham et al., 2009; Yoshizaki et al., 2009, 2010), probably through the negative regulation of the nuclear factor-κB pathway (Yeung et al., 2004). In addi-

tion, SIRT1 activity has a strong influence on cell proliferation, apoptosis, and cancer. Although the data in vitro is controversial, the work on genetically engineered mouse models indicates that enhanced SIRT1 activity would be protective against the development of some types of cancer (Herranz et al., 2010). SIRT1 may also be of interest in the central nervous system. It was recently proven that SIRT1 has key roles modulating cognitive function and synaptic plasticity (Gao et al., 2010; Michán et al., 2010). In addition, there is evidence that enhanced SIRT1 activity could be protective in conditions such as neurodegeneration, Alzheimer's disease, and amyotrophic lateral sclerosis (Araki et al., 2004; Chen et al., 2005a; Kim et al., 2007a; Donmez et al., 2010). Because we focus mainly on the metabolic impact of SIRT1, we refer the reader to other reviews for discussion of these other fields of action for SIRT1 (Finkel et al., 2009; Herranz and Serrano, 2010; Guarente, 2011).

The identification of p53 as a SIRT1 substrate enlightened the scientific community on the versatility of SIRT1, which was until then largely considered a histone deacetylase. Two different laboratories simultaneously reported how SIRT1 interacts with and deacetylates p53 (Luo et al., 2001; Vaziri et al., 2001). Although p53 can be acetylated in up to six residues, SIRT1 seems to preferentially deacetylate Lys^{379} (human Lys^{382}). The deacetylation of p53 by SIRT1 attenuated its activity on the p21 promoter and inhibited p53-dependent apoptosis (Luo et al., 2001; Vaziri et al., 2001). This link between p53 and SIRT1 activities led to the premature hypothesis that SIRT1 inhibition could lead to tumor suppression and, vice versa, that SIRT1 activation would promote tumor formation. However, SIRT1 transgenic models challenge this hypothesis and point out that SIRT1 activation actually suppresses tumor formation (for review, see (Herranz and Serrano, 2010). This discrepancy might stem from different, yet unresolved, issues. For example, it is not clear whether physiological deacetylation of p53 in situations of higher SIRT1 activity are modulated via direct deacetylation or take place indirectly through changes in other cellular processes, such as affecting its interaction with p300 (Bouras et al., 2005). In addition, as described later in this section for other transcriptional regulators, p53 activity depends not only on the modulation of its acetylation levels but also on many other post-translational modifications, which create a "bar-code"-like situation determining specific activity (Murray-Zmijewski et al., 2008). A very nice example about why p53 "activation" should be reworded as "specification" is provided by the actions of p53 on mitochondrial metabolism. In general, p53 activation has been linked to enhanced mitochondrial oxidation, while p53 deletion is associated with defective mitochondrial respiratory rates (Zhou et al., 2003; Matoba et al., 2006; Saleem et al., 2009). However, activation of p53 in the context of DNA damage can paradoxically lead to decreased mitochondrial biogenesis (Sahin et al., 2011). This highlights how the activity of transcription factors can be channelled in different ways depending on the biological context of their activation and, probably, on a differential post-translational modification "bar-code."

The Forkhead-O-box (FOXO) family of transcription factors constitute another example of how SIRT1 channels the activity of transcriptional regulators toward specific targets FOXOs are key regulators of lipid metabolism, stress resistance, and apoptosis (Gross et al., 2008), and SIRT1 has been shown to interact with and deacetylate the FOXO family of transcription factors (Brunet et al., 2004; Motta et al., 2004). It is noteworthy that deacetylation of FOXO3 by SIRT1 inhibited its activity on apoptosis-related gene expression but drove its actions toward the induction of oxidative stress resistance genes (Brunet et al., 2004). In addition, SIRT1-mediated FOXO deacetylation also enhances autophagy (Hariharan et al., 2010). This is in line with the hypothesis that activation of SIRT1 allows the cell to adapt to situations of energy stress. It is noteworthy that SIRT1 activity, as well as FOXO1 and FOXO3 deacetylation, is prompted by situations of oxidative stress, energy stress, and fasting (Brunet et al., 2004; Cantó et al., 2009, 2010). Also remarkable is that FOXO and SIRT1 orthologs in lower eukaryotes have both been linked to lifespan extension (Greer and Brunet, 2008; Cantó and Auwerx, 2009). All together, the correlative activation and effects on lifespan, metabolism, and adaptation to energy stress suggest that SIRT1 and FOXO activities might be linked mechanistically through this SIRT1-mediated deacetylation. When the acetylatable residues in FOXO are mutated to mimic a constant acetylated state (Lys→Gln), FOXO becomes more sensitive to Akt-mediated phosphorylation and nuclear exclusion (Qiang et al., 2010). Conversely, when the mutations mimic the deacetylated state (Lys→Arg), FOXO is retained in the nucleus (Qiang et al., 2010). These mutants further confirmed that FOXO deacetylation is required for the effects of oxidative stress, FOXO nuclear trapping, and the induction of stress resistance gene expression (Qiang et al., 2010). The mere coexistence of FOXOs and SIRT1 in the nucleus is insufficient to promote their interaction in the absence of energy or oxidative stresses (Brunet et al., 2004), underscoring the necessity of an additional stress-derived signal to trigger their functional interaction.

A similar case can be made for the transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)γ coactivator 1α (PGC-1α). PGC-1α acts as a master regulator of mitochondrial biogenesis in vertebrates (Puigserver et al., 1998; Rodgers et al., 2005) and orchestrates a constellation of transcription factors (such as the estrogen-related receptors, the nuclear respiratory factors 1 and 2, or PPARs) to induce mitochondrial gene expression (Wu et al., 1999; Rodgers et al., 2005). Seminal work by the Puigserver laboratory illustrated how PGC-1α is acetylated and how deacetylation of PGC-1α by SIRT1 is a key event required for its activation (Rodgers et al., 2005; Lerin et al., 2006). In situations of energy stress or SIRT1

activation, PGC-1α is prominently deacetylated and activated (Rodgers et al., 2005; Gerhart-Hines et al., 2007; Cantó et al., 2009). PGC-1α can be acetylated in up to 13 lysine residues (Rodgers et al., 2005), and although there is still not a clear idea on the differential contribution of each residue, mutation of all 13 lysines into arginine (mimicking constant deacetylation), constitutively activates PGC-1α (Rodgers et al., 2005). PGC-1α is a nuclear protein, and therefore may coexist with SIRT1 in the nucleus. However, as happened with the FOXOs, only upon energy stress is physiological activation of PGC-1α by SIRT1 prominent, indicating that additional signals are required to prompt SIRT1-mediated PGC-1α deacetylation. The mechanism by which this specification happens has recently been unveiled. Energy or nutrient stress is generally translated in imbalanced AMP/ATP ratios (Hardie, 2007). Whenever there is an increase in the AMP/ATP ratio or ADP/ATP ratio, be it by enhanced ATP consumption or defective ATP synthesis, the enzymatic activity of the AMP-activated protein kinase (AMPK) is enhanced (for a mechanistic review, see Hardie, 2007). PGC-1α is a substrate for AMPK phosphorylation, leading to its activation (Jäger et al., 2007), even though the manner by which this phosphorylation activates PGC-1α remains elusive. Cantó et al. (2009) described how the phosphorylation of PGC-1α by AMPK in situations of energy stress is required to prime it for subsequent deacetylation and activation by SIRT1. This AMPK/SIRT1/PGC-1α signaling pathway is, furthermore, the mechanism by which several hormones enhance mitochondrial metabolism, as is the case for adiponectin (Iwabu et al., 2010), leptin (Li et al., 2011), or fibroblast growth factor 21 (Chau et al., 2010). It is noteworthy that SIRT1 can deacetylate nonphosphorylated PGC-1α in vitro (Nemoto et al., 2005), indicating that the cellular context poses some constraints for this reaction/interaction to happen. It is likely that AMPK-mediated phosphorylation of PGC-1α modifies the nuclear localization of PGC-1α and/or allows the interaction with third-party proteins that reinforce the stability of the SIRT1 and PGC-1α interaction. It is also tempting to hypothesize that a similar mechanism explains why, despite their coexistence in the nucleus, FOXOs are only interacting with SIRT1 in situations of energy stress. In fact, FOXOs are also phosphorylated in response to energy stress by AMPK (Greer et al., 2007). Further experiments will have to verify whether, as is the case for PGC-1α, this phosphorylation by AMPK converges with SIRT1 deacetylation to target FOXO toward specific gene sets, such as those related to protection against oxidative stress. In all, the AMPK and SIRT1 signaling pathway highlights the interactive nature of different post-translational modifications on transcriptional activators, such as PGC-1α and FOXO, that allow them to select specific downstream pathways.

Both PGC-1α and FOXOs are transcription factors that, upon deacetylation by SIRT1, will enhance lipid catabolism and mitochondrial respiration. However, SIRT1 can also directly block lipid anabolism by interfering with PPARγ and liver X receptor (LXR) signaling. PPARγ is a nuclear receptor that is mainly expressed in white adipose tissue and plays key roles in adipocyte differentiation, lipid synthesis, and storage (Heikkinen et al., 2007). SIRT1 represses PPARγ activity, even though it is not clear whether this is mediated by acetylation-related events in the PPARγ protein (Picard et al., 2004). A recent report indicates that PPARγ can be directly deacetylated by SIRT1, although the relevance of this acetylation for PPARγ activity is not yet known (Han et al., 2010). It is known, however, that the repressive effect of SIRT1 requires the formation of a corepressor complex that also involves the nuclear receptor corepressor 1 (NCoR1) (Picard et al., 2004). Hence, during fasting, SIRT1 associates with NCoR1 and represses PPARγ function, favoring fat mobilization instead of storage. This work also highlights how many actions of SIRT1 are determined by its interaction with specific protein complexes. Therefore, understanding the dynamics of how SIRT1 merges into different protein complexes will be essential to understanding how to drive SIRT1 toward specific sets of actions.

Although PPARγ is a major controller of lipid anabolism in adipose tissue, other nuclear receptors can also perform similar functions in other tissues. For example, LXRα and -β are well known for their ability to sense oxysterols and regulate genes that decrease total body cholesterol levels (Kalaany and Mangelsdorf, 2006). LXRs, however, are also potent stimulators of lipid anabolism through the induction of the sterol regulatory element binding protein-1c (SREBP-1c) and its downstream targets, stearoyl-CoA desaturase 1, acyl-CoA carboxylase, and fatty acid synthase (Kalaany and Mangelsdorf, 2006). LXRs are acetylated at Lys^{432} in LXRα and Lys^{433} in LXRβ (Li et al., 2007). Upon LXR activation, LXR interacts with SIRT1, which then removes their acetyl groups (Li et al., 2007). Deacetylation of LXR increases its transcriptional activity, even though the deacetylated lysine residue of LXR makes it also more prone to ubiquitination and degradation (Li et al., 2007). The key role of SIRT1 in the modulation of LXR activity fits with the impaired cholesterol homeostasis and hepatic cholesterol accumulation observed in SIRT1-null mice. The impact of SIRT1 on cholesterol homeostasis was further supported by other studies showing how the absence of SIRT1 reduced the expression of CYP7A1, the rate-limiting enzyme in the bile acid synthesis (Rodgers and Puigserver, 2007), even though whether this phenomenon is strictly LXR-dependent is currently unclear. Given that SIRT1 stimulates both macrophage cholesterol efflux to the liver (Li et al., 2007) and the hepatic conversion of cholesterol into bile acids potentially through LXR (Rodgers and Puigserver, 2007), the SIRT1-LXR pathway seems therefore to be important for reverse cholesterol transport. The effects of LXR and SIRT1 on lipid homeostasis, however, are more conflictive. In theory, LXR activation by SIRT1 should increase liver triglyceride accumulation. Liver-specific deletion of SIRT1, however, induces hepatic steatosis, whereas gain of SIRT1 function is protective (see

section III). Although the latter observation fits with the higher oxidative metabolism expected after SIRT1 activation, it seems incompatible with LXR activation. A likely explanation is that SIRT1 activation not only deacetylates LXR but also its major mediator in the induction of triglyceride synthesis, SREBP-1c (Ponugoti et al., 2010; Walker et al., 2010). In fact, SREBP-1c is stabilized by p300-mediated acetylation (Giandomenico et al., 2003), and the deacetylation of SREBP-1c by SIRT1 at Lys^{289} and Lys^{309} makes the protein prone to ubiquitin-mediated degradation (Giandomenico et al., 2003; Ponugoti et al., 2010). The abrogation of SREBP-1c activity would hence allow SIRT1 to promote beneficial effects on cholesterol metabolism through activation of LXR in the absence of detrimental effects on liver lipid accumulation.

Although the modulation of all the above transcription factors mainly influences lipid metabolism in white adipose tissue and liver, SIRT1 also modulates carbohydrate metabolism via deacetylation of other transcription factors. The liver maintains blood glucose levels during fasting through gluconeogenesis. A key transcriptional regulator of gluconeogenic gene expression is the cAMP response element-binding (CREB) protein, whose activity is largely controlled by the binding of its coactivator CREB-regulated transcriptional coactivators (CRTCs) (Altarejos and Montminy, 2011). Among the three members of the CRTC family, CRTC-2 has been reported to be key to properly induce gluconeogenic gene expression (Altarejos and Montminy, 2011). In the fed state, CRTC-2 is hyperphosphorylated, probably by the salt-inducible kinase 2, which sequesters it in the cytosol by avid binding to 14-3-3 platform proteins (Screaton et al., 2004; Koo et al., 2005). Upon exposure to cAMP or calcium signals during fasting, CRTC2 is dephosphorylated by calcineurin, released from 14-3-3 and therefore able to translocate to the nucleus, where it binds and activates CREB on relevant gluconeogenic gene promoters, such as those of the phosphoenolpyruvate carboxykinase and glucose 6-phosphatase genes (Screaton et al., 2004). The coactivation of CREB by CRTC2, however, is only transient during the early stages of fasting (Liu et al., 2008). Upon prolonged fasting, and coinciding with the hepatic increase in NAD^+ and SIRT1 activation, CRTC2 activity decreases (Rodgers et al., 2005; Liu et al., 2008). SIRT1 activation in fact deacetylates CRTC2 at Lys^{628}, leading to the COP-1-mediated ubiquitylation and proteasome-dependent degradation of CRTC2 (Liu et al., 2008). Because gluconeogenesis consumes ATP, this action of SIRT1 may attenuate gluconeogenesis in an effort to prevent premature energy depletion upon protracted fasting. It is noteworthy that restraining the activity of orthologs of CRTC and CREB in *C. elegans* prolongs lifespan (Mair et al., 2011), which suggest that decreased levels of CRTCs after deacetylation may be one way by which SIRT1 orthologs may affect lifespan in worms.

All together, the ensemble of metabolic transcriptional regulators directly affected by SIRT1 enables it to orchestrate cellular and whole-body metabolism to extract energy from noncarbohydrate sources, especially by mitochondrial respiration-based routes (Fig. 3). This is in line with the

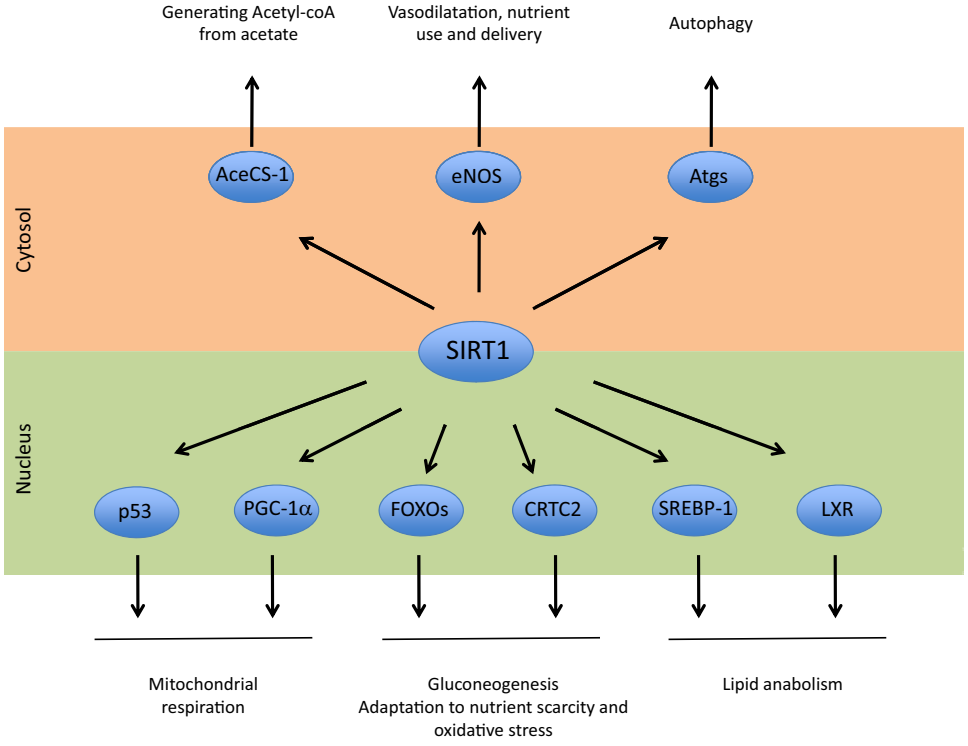


FIG. 3. SIRT1 metabolic targets. SIRT1 deacetylates a large array of protein targets involved in metabolic regulation. The bottom part of the figure highlights nuclear targets implicated in transcriptional metabolic adaptations. SIRT1's cytosolic targets are illustrated in the top part. The full names for the abbreviations can be found in the main text. Atgs, autophagy-related proteins.

observation that SIRT1 is activated in situations of nutrient deprivation and energy stress.

2. Cytosolic Targets. As mentioned before, SIRT1 is also present in the cytosol in many cell types, especially when insulin signals are lacking (Tanno et al., 2007). This suggests that SIRT1 also modifies the activity of cytosolic enzymes through direct deacetylation (Fig. 3).

Initial evidence for the existence cytosolic SIRT1 targets came from the discovery that the cytosolic acetyl-CoA synthetase 1 (AceCS-1) enzyme is deacetylated by SIRT1, but not by other sirtuins (Hallows et al., 2006). AceCS-1 can generate acetyl-CoA from acetate. Although this enzyme has a key role in bacterial energy metabolism, the impact of acetate metabolism and AceCS-1 on mammalian whole-body metabolism is not yet clear. AceCS-1 is acetylated on Lys^{661} in the catalytic domain (Hallows et al., 2006). The activity of AceCS-1 is almost 50 times lower in its acetylated state (Hallows et al., 2006), and SIRT1 deacetylation, therefore, serves as an activation switch. The dynamic regulation of AceCS-1 acetylation in response to physiological events, however, has not yet been explored.

Another cytoplasmic enzyme deacetylated by SIRT1 is the endothelial nitric-oxide synthase (eNOS) (Mattagajasingh et al., 2007). SIRT1 deacetylates Lys^{496} and Lys^{506} in the calmodulin-binding domain of eNOS, thereby activating it to boost endothelial nitric oxide levels (Mattagajasingh et al., 2007). Endothelial inhibition of SIRT1 leads to inefficient endothelium-dependent vasodilation (Mattagajasingh et al., 2007), a process key for proper nutrient supply to tissues. The activation of eNOS by SIRT1 could hence be a mechanism by which nutrient scarcity increases energy delivery into tissues. It is noteworthy that impaired eNOS function has major consequences on whole-body metabolism, since it affects peripheral glucose uptake (Kapur et al., 1997; Li et al., 2004) and mitochondrial biogenesis (Nisoli et al., 2003; Nisoli et al., 2005; Le Gouill et al., 2007).

The impact of SIRT1 in metabolic cytosolic processes was further underscored by the discovery that SIRT1 forms molecular complexes with critical components of the autophagy machinery, including Atg5, Atg7, and Atg8 (Lee et al., 2008). SIRT1 deacetylates these proteins in an NAD$^+$-dependent manner, even though the substrate residues and the consequences of this deacetylation have not yet been fully elucidated (Lee et al., 2008). Autophagy during starvation is hence impeded in embryonic fibroblasts of SIRT1(−/−) mice and leads to the accumulation of damaged organelles, especially mitochondria (Lee et al., 2008). This phenomenon contributes to the fact that impaired SIRT1 activity systematically correlates with deficiencies in energy metabolism and fits with the hypothesis of SIRT1 being a master metabolic switch driving the cell to obtain energy from noncarbohydrate energy sources.

III. Sirtuin 1 and Metabolic Disease: Evidence from Mice Models

The attractive effects of SIRT1 orthologs in lower organisms, as well as at the cellular and molecular level in mammalian cells, prompted the generation of mouse models to evaluate the impact of SIRT1 on whole body metabolism. This goal proved more difficult to achieve than expected, because inbred germline SIRT1-deficient mice have high prenatal death rates (McBurney et al., 2003). The very few pups that were born presented severe neurological and cardiac defects, resulting in early postnatal death (McBurney et al., 2003). Outbred mice with the SIRT1 mutation, however, were viable (McBurney et al., 2003). From a metabolic perspective, SIRT1 knockout mice were metabolically inefficient and showed impaired calorie restriction-induced effects on metabolism and longevity (Boily et al., 2008). The outbred mouse line, however, is not ideal for metabolic studies, and inducible models will be required to evaluate how whole-body deletion of the *SIRT1* gene will affect global metabolism.

In the meantime, however, several tissue-specific somatic SIRT1-deficient mouse models already provided ample evidence that most of the in vitro biology of SIRT1 translates into an in vivo context. Most of the metabolic work on SIRT1 has been focused on muscle cells and hepatocytes. Although most evidence in cultured muscle cells indicates a key role for SIRT1 in the modulation of mitochondrial metabolism (Gerhart-Hines et al., 2007; Cantó et al., 2009), initial studies in the muscle-specific SIRT1 knockout mice indicate that SIRT1 is not required for exercise-induced deacetylation of PGC-1α or mitochondrial biogenesis in skeletal muscle (Philp et al., 2011). Complementary mice models and physiological challenges will be required to help clarify the role of SIRT1 in skeletal muscle. In contrast to the situation in muscle, many different studies have focused on liver-specific SIRT1 gene deletion (Chen et al., 2008; Purushotham et al., 2009; Wang et al., 2010). The lack of SIRT1 in liver does not induce an overt phenotype on chow diet, and these mice respond normally to calorie restriction (Chen et al., 2008). However, diametrically opposite results became apparent when the physiological impact of high-fat diet in two independent liver-specific SIRT1-deficient mice lines was evaluated. Whereas Chen et al. (2008) found that the liver-specific SIRT1-null mice gained less weight upon high-fat feeding, maintained better glucose tolerance, and were protected against hepatic steatosis, Purushotham et al. (2009) reported that liver SIRT1 deletion increased susceptibility to hepatic steatosis and body weight gain upon high-fat feeding. Furthermore, Purushotham et al. (2009) also reported that the lack of SIRT1 in liver also enhanced hepatic triglyceride accumulation upon fasting. A subsequent study, using yet another hepatocyte SIRT1 knockout mouse line, reported prominent liver steatosis in chow-fed mice at young ages that worsened with age (Wang et al., 2010). It must be noted that germline heterozygous SIRT1-deficient

mice also show a marked tendency toward liver lipid accumulation (Xu et al., 2010). This conclusion would also be totally in line with the in vitro observations suggesting that SIRT1 enhances fat oxidation and that SIRT1 activity down-regulates SREBP-1c, a master controller of fatty acid synthesis (Rodgers and Puigserver, 2007; Ponugoti et al., 2010; Walker et al., 2010).

Other investigators have analyzed the role of SIRT1 in the liver by knocking its expression down through tail vein injection of adenoviruses carrying a SIRT1 short hairpin RNAs. Strikingly, no alterations in triglyceride accumulation were observed in livers acutely depleted of SIRT1 (Rodgers and Puigserver, 2007). Instead, glucose homeostasis was severely impaired and gluconeogenic capacity was defective upon SIRT1 reduction (Rodgers and Puigserver, 2007). This role of SIRT1 in glucose homeostasis, however, was not observed in any of the above-mentioned hepatic SIRT1-deficient mouse lines. This might indicate either that the adenoviral short hairpin RNA delivery is causing additional effects or that the defects in glucose homeostasis upon acute decrease in SIRT1 levels are somehow compensated in the liver-specific SIRT1 knockout and the germline heterozygotic SIRT1-deficient mice, which have chronic reductions in hepatic SIRT1 expression. A role of SIRT1 in gluconeogenesis, furthermore, is highly debated. From one side, it is speculated that SIRT1 enhances gluconeogenesis via the deacetylation and activation of PGC-1α, which, in turn, would coactivate CREB on the promoters of gluconeogenic genes (Herzig et al., 2001; Yoon et al., 2001). However, although there is no doubt that artifactual overexpression of PGC-1α enhances gluconeogenic gene expression in liver, it must be pointed out that the evidence indicating that physiological modulation of PGC-1α activity is participating in gluconeogenesis is weak (Herzog et al., 2004). On the other hand, a plethora of scenarios have illustrated how SIRT1 activation in liver is not per se associated with enhanced glucose production but rather to attenuated gluconeogenic rates (summarized in Cantó and Auwerx, 2010).

The role of SIRT1 in pancreatic function has also been characterized in genetically engineered mouse models. Studies in outbred SIRT1 knockout mice indicated that SIRT1 deficiency blunts pancreatic insulin secretion (Bordone et al., 2006). The etiology of this defect is not entirely clear, even though it was proposed to have come from the negative regulation that SIRT1 exerts on uncoupling protein 2 (UCP2) expression (Bordone et al., 2006). The lack of SIRT1 leads to higher UCP2 levels, which alter the ability of glucose to modulate ADP/ATP ratios in pancreatic β cells and trigger insulin release (Zhang et al., 2001). The influence of SIRT1 on insulin release was confirmed in mice that specifically overexpressed SIRT1 in pancreatic β cells, which manifested enhanced glucose-induced insulin secretion (Moynihan et al., 2005). This study further certified how UCP2 is negatively regulated by SIRT1, therefore allowing better coupling and ATP production in response to high glucose in the SIRT1-overexpressing mice (Moynihan et al., 2005). However, SIRT1 also enhanced insulin secretion upon artificial depolarization with KCl, indicating that SIRT1 alters insulin release by additional mechanisms downstream of depolarization and independent of UCP2 (Moynihan et al., 2005). It is noteworthy that the beneficial effects of β-cell SIRT1 overexpression were restricted to young mice and lost upon aging (Ramsey et al., 2008). Although the explanation for this phenomenon is not clear yet, it might originate in the NAD^+-dependence of SIRT1, because aging is known to decrease NAD^+ levels in rodent tissues (Braidy et al., 2011). Therefore, it is likely that the reduction in NAD^+ limits SIRT1 activity during aging, attenuating its beneficial effects on insulin secretion and glucose homeostasis.

The functions of SIRT1 in central nervous system control of metabolism have also not escaped attention. The neuronal deletion of SIRT1 does not affect brain development but reduces body size as a consequence of a specific deficiency in pituitary growth hormone production (Cohen et al., 2009). Although mice with the neuronal deletion of SIRT1 showed no major differences in glucose tolerance compared with wild-type littermates on both chow and high-fat diets at young age, defects in glucose homeostasis were exacerbated upon aging (Cohen et al., 2009). The reasons for these particular phenotypes have not been elucidated. Conversely, brain-specific overexpression of SIRT1 did not result in a major phenotypic change in the basal state (Satoh et al., 2010). Based on these reports, no clear picture of how central nervous system SIRT1 activity influences global metabolism has emerged as yet.

Although other tissue-specific mouse models are being generated at present, the role of SIRT1 in metabolism has also been studied using whole-body gain-of-function SIRT1 mice. The first SIRT1 gain-of-function mouse model displayed several phenotypes that resembled calorie-restricted mice; the animals were leaner, metabolically more active, and had increased glucose tolerance suggestive of insulin sensitization (Bordone et al., 2007). The other two mouse lines that overexpress SIRT1 further explored the impact of diet- and genetics-induced obesity, both concluding that mild SIRT1 overexpression protects against the development of hyperglycemia, metabolic disease, and fatty liver (Banks et al., 2008; Pfluger et al., 2008). The above results would be in line with the observations in liver SIRT1 knockout mice reporting higher hepatic fat accumulation (Purushotham et al., 2009; Wang et al., 2010). It is noteworthy that although SIRT1 transgenic mice were protected against the onset of age-related diseases, such as cancer and metabolic diseases, they did not live longer (Herranz et al., 2010). These data further emphasize that no firm evidence has yet been found indicating that SIRT1 influences lifespan in mammals.

All together, the information provided by genetically engineered mouse models supports the notion that SIRT1 activation has metabolic benefits. In the next section, we describe the efficiency, specificity, and results obtained

from different strategies aimed at artificially activating SIRT1.

IV. Physiological and Pharmacological Modulation of Sirt1 Activity

A. The Modulation of Sirtuin 1 Expression

A first line of action when aiming to enhance the biological action of a protein is to increase its expression levels. Indeed, the simple overexpression of SIRT1 in cells and tissues is enough to increase SIRT1 activity (Rodgers et al., 2005; Rodgers and Puigserver, 2007; Banks et al., 2008), indicating that NAD^+ might not be limiting for SIRT1 activity in basal conditions. Strikingly, the elucidation of the transcriptional mechanisms controlling SIRT1 expression has only recently begun. We focus here on the mechanisms related to the metabolic and redox control of SIRT1 expression (Fig. 4).

SIRT1 expression is generally higher in situations of low nutrient availability and endurance exercise (Nemoto et al., 2004). In mice and humans, SIRT1 expression was shown to correlate with higher expression of nuclear encoded mitochondrial genes and energy expenditure (Lagouge et al., 2006; Rutanen et al., 2010). The earliest studies on *SIRT1* gene expression aimed to understand how SIRT1 mRNA levels increase in response to nutrient deprivation. In these studies, FOXO3a, a member of the FOXO family of transcription factors, was shown to indirectly increase rodent SIRT1 transcription through its proximal promoter. It is noteworthy that FOXO3a modulates SIRT1 activity via its interaction with p53, and this interaction was shown to be nutrient-dependent (Nemoto et al., 2004). In the absence of this interaction, p53 acts as a repressor of the SIRT1 promoter (Nemoto et al., 2004). Therefore, a model was build in which, under normal conditions, p53 represses SIRT1, and, upon nutrient starvation, activated FOXO3a interacts with p53 and relieves the inhibition of SIRT1 transcription, probably by changing the balance of coactivators/corepressors on the SIRT1 promoter. It is noteworthy that this inter-relation highlights how SIRT1, p53, and FOXO3a activities are interconnected with feedback loops: SIRT1 and p53 negatively regulate each other, via deacetylation (see section II.C) and transcriptional events, respectively, generating a homeostatic loop balancing both activities. Conversely, SIRT1-mediated deacetylation of FOXO3a enhances FOXO3a activity, and this is further amplified by the FOXO3a-mediated induction of SIRT1 expression. It is noteworthy that the rat SIRT1 promoter contains multiple FOXO1 core binding motifs and a forkhead-like consensus binding site, which enable FOXO1 to directly activate SIRT1 transcription, which is in contrast to the indirect effects of FOXO3a (Xiong et al., 2011). As happened with FOXO3a, FOXO1 and SIRT1 would create a feed-forward loop in which FOXO1 activation by SIRT1-mediated deacetylation amplifies SIRT1 expression and activity. It will be interesting to elucidate whether these FOXO1 binding sites are evolutionarily conserved and how this feed-forward loop is integrated with other regulatory mechanisms of SIRT1 transcription. Likewise, it will be crucial to understand the mechanisms by which FOXOs are driven to the SIRT1 promoter upon glucose deprivation. It is of note that FOXOs are activated by AMPK (Greer et al., 2007), which is known to increase SIRT1 expression (Suwa et al., 2011).

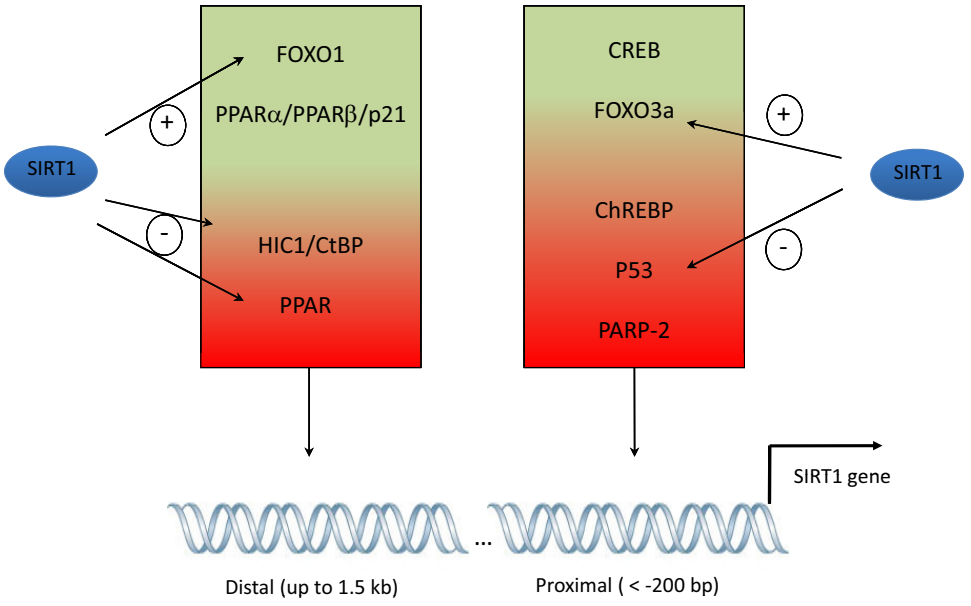


FIG. 4. Transcriptional regulation of the SIRT1 gene. Many transcription factors influence the transcriptional activity by acting on both the proximal and distal regions of the SIRT1 promotor. Transcriptional regulators in the green part of the boxes positively regulate SIRT1 gene expression, whereas those in the red part of the boxes act as negative regulators. It is noteworthy that the SIRT1 protein can create many feed-forward loops by deacetylating and enhancing the activity of some positive regulators (FOXOs) while deacetylating and/or inactivating repressor complexes (p53, PPARγ, HIC1/CtBP). Full names for the abbreviations can be found in the text.

SIRT1 can also promote another negative-feedback loop on its own promoter through hypermethylated in cancer 1 (HIC1) (Chen et al., 2005b). HIC1 naturally forms a transcriptional corepressor complex with SIRT1, which binds directly to the SIRT1 promoter and down-regulates its transcription (Chen et al., 2005). It is noteworthy that the repressive activity of HIC1 on the SIRT1 promoter is regulated by its association with CtBP, a sensor of energy/redox stress (Zhang et al., 2007). The binding of CtBP to transcriptional repressors such as HIC1 is enhanced by NADH (Zhang et al., 2002). Therefore, in situations of low glycolytic rates, such as those seen upon 2-deoxyglucose treatment, NADH will decrease, destabilizing CtBP/HIC1/SIRT1 inhibitory complexes, thereby allowing the induction of SIRT1 mRNA levels. The increase in SIRT1 levels would therefore be a way for metabolic adaptation toward the utilization of noncarbohydrate energy sources.

Although SIRT1 content is generally higher upon nutrient deprivation, it is also known that SIRT1 is reduced in obese mice and humans (Coste et al., 2008; Costa Cdos et al., 2010). It was therefore interesting to find how PPARγ activation down-regulates SIRT1 expression (Han et al., 2010). The distal SIRT1 promoter contains PPAR-response elements (PPREs) (Han et al., 2010; Hayashida et al., 2010), even though a thorough mapping of the sites and their evolutionary conservation is lacking. Upon activation, PPARγ can bind to and repress the SIRT1 promoter and, as such, provide a mechanism by which SIRT1 expression can be reduced in situations of nutrient overload. It is noteworthy that PPREs can bind also other PPARs. Therefore, it would be expected that other PPARs also regulate the SIRT1 promoter. Confirming this speculation, PPARα or PPARβ/δ activation by synthetic ligands enhances SIRT1 expression (Hayashida et al., 2010; Okazaki et al., 2010). Although the mechanism through which PPARα regulates the SIRT1 promoter remains unclear, the actions of PPARβ/δ are not mediated by its direct binding to the PPREs, but rather involve binding to p21, which has a conserved binding site in the proximal human SIRT1 promoter (Okazaki et al., 2010). All together, it is interesting that the PPARs, which act as lipid sensors (Schoonjans et al., 1996) control SIRT1 activity, with PPARγ, related to lipid anabolism, inhibiting SIRT1 expression, whereas PPARα and PPARβ/δ, both linked to fatty acid oxidation, increase SIRT1 mRNA levels. Therefore, the differential sensing of lipid species might be essential to understanding how lipid metabolism can influence SIRT1 expression and drive adaptations toward lipid anabolic or catabolic pathways, and future work addressing the distinct effects of the different PPARs is warranted.

Another recent interesting finding about the transcriptional regulation of SIRT1 expression came from the studies of a nonsirtuin NAD^+ consumer, the poly(ADP-ribose) polymerase (PARP)-2 protein. PARP-2 is a member of a large family of PARP proteins (see section IV.D.2.a). Although in general PARP-2 has been mainly considered to be part of the DNA damage-repair machinery, it has been shown that PARP-2 also acts as a transcriptional modulator (Bai et al., 2007). PARP-2 enhances the transcriptional activity of PPARγ, but not that of PPARα or PPARβ/δ, therefore favoring adipocyte differentiation and fat storage (Bai et al., 2007). In contrast, PARP-2 decreases the activity of the SIRT1 promoter by directly binding to the proximal −91-base pair region (Bai et al., 2011a). Consistent with this, decreased PARP-2 levels enhanced SIRT1 gene expression, which translated into higher SIRT1 activity (Bai et al., 2011a). At the whole-body level, PARP-2 deletion mimics all the features of SIRT1 activation, such as higher mitochondrial content, enhanced oxidative metabolism, and protection against diet-induced obesity and insulin resistance (Bai et al., 2011a). It will be interesting to elucidate how PARP-2 influences SIRT1 transcription and whether this may involve the poly-ADP-ribosylation of other proteins near the SIRT1 promoter. In addition, how PARP-2 can both activate and repress transcription requires further study.

Although most of the above examples provide a number of candidate transcription factors that influence SIRT1 transcription in vitro, few of them have been clearly linked to the physiological modulation of SIRT1 expression by hormones and the feeding/fasting cycles in vivo. Noriega et al. (2011) has identified how, during feeding, the carbohydrate response element-binding protein (ChREBP) directly binds to a composite response element in the proximal SIRT1 promoter and represses its transcription. Upon fasting, ChREBP is translocated to the cytosol, and its binding site on the promoter is now liberated. This enables CREB, whose activity is enhanced by the cAMP signal generated by the fasting hormones, glucagon and norepinehrine, to bind and enhance *SIRT1* gene expression (Noriega et al., 2011). This way, the opposite effects of CREB and ChREBP on SIRT1 transcription constitute the first established mechanism for the regulation of SIRT1 expression in response to physiological fasting/feeding cycles. This also highlights that the proximal SIRT1 promoter is a hot spot for its physiological regulation (Fig. 4) and further emphasizes that SIRT1 is a crucial metabolic checkpoint connecting the energetic status with transcriptional programs downstream of SIRT1.

Finally, a interesting mechanism regulating SIRT1 expression is the control by microRNAs (miRNAs), which emerge as key controllers of global gene expression (Neilson and Sharp, 2008). miRNAs bind to the 3′-untranslated region of target mRNAs and inhibit their expression by causing mRNA cleavage or inhibition of translation (Neilson and Sharp, 2008). It is assumed that approximately 30% of all human genes are regulated by miRNAs (Neilson and Sharp, 2008). It has been reported that miRNA-34a targets hepatic SIRT1 and negatively correlates with SIRT1 expression (Yamakuchi et al., 2008; Lee et al., 2010). miRNA-34a binds to the 3′-untranslated region of SIRT1 mRNA in a partial complementary manner and represses its translation (Yamakuchi et al., 2008; Lee et al., 2010). miRNA-34a levels are consistently elevated in

the livers from diet-induced and genetically obese mice (Lee et al., 2010). Remarkably, p53, which negatively regulates SIRT1 expression directly, also induces miRNA-34a, providing an additional mechanism to ensure SIRT1 repression upon p53 activation (Yamakuchi and Lowenstein, 2009). Other miRNAs have been reported to also affect SIRT1 expression in different tissues, such as miRNA-132, which down-regulates SIRT1 expression in adipose tissue, prompting inflammatory responses (Strum et al., 2009). Therefore, miRNAs are providing a completely new level for the regulation of SIRT1 levels that we are only beginning to grasp.

Together, these mechanisms illustrate the complexity of the regulation of SIRT1 at the level of its expression. It is noteworthy that most of the transcriptional regulators described are also substrates for SIRT1, which illustrates the intricate nature of SIRT1 regulation and how it is driven by multiple regulatory loops. However, multiple interactions between proteins and the multifunctionality of the individual proteins involved make it difficult to predict how pharmacological targeting of one of the players will affect the others. Although the identification of novel players will certainly contribute to our understanding of SIRT1 transcriptional regulation, it is understandable that, at this point, alternative strategies to enhance SIRT1 activity are also of interest. Such strategies will be described in the next sections.

B. Post-Translational Modifications

The activity of SIRT1, like that of most enzymes, is also modulated by a number of post-translational modifications. The first report indicating this possibility was the identification of SIRT1 as a nuclear phosphoprotein in a large screening using mass spectrometry (Beausoleil et al., 2004). Although two phosphoresidues, Ser^{27} and Ser^{47}, were identified, their function has not yet been explored. Subsequent efforts identified up to 13 phosphorylatable residues, including the two found previously (Sasaki et al., 2008). Dephosphorylated SIRT1 was less active than the phosphorylated form (Sasaki et al., 2008). Among these residues, Thr^{530} and Ser^{540} were phosphorylated by cyclinB/cdk1, and their mutation resulted in aberrant cell proliferation and cell cycle profiles that could not be explained by changes in SIRT1 stability but rather resulted from lower SIRT1 activity (Sasaki et al., 2008). Another report indicated that JNK1 can also phosphorylate SIRT1 in three residues, Ser^{27}, Ser^{47}, and Thr^{530}, in response to oxidative stress promoted by H_2O_2 or anisomycin (Nasrin et al., 2009). In agreement with the previous report, phosphorylation of these sites by JNK1 increased nuclear localization of SIRT1 and its activity (Nasrin et al., 2009). Surprisingly, it also seemed that JNK1 phosphorylation oriented SIRT1 activity toward specific substrates, because it triggered the deacetylation of histone H3 but not p53 (Nasrin et al., 2009). Paradoxically, JNK1 activity is induced in situations of obesity and metabolic disease (Hirosumi et al., 2002), where SIRT1 activity is decreased (Coste et al., 2008). A constellation of other kinases, including casein kinase II (Kang et al., 2009; Zschoernig and Mahlknecht, 2009), the dual-specificity tyrosine phosphorylation-regulated kinases (Guo et al., 2010), and the mammalian sterile 20-like kinase 1 (Yuan et al., 2011a), have also been suggested to phosphorylate SIRT1. Although these data indicate that the activity of SIRT1 might be modulated through the phosphorylation of the above-mentioned residues by multiple kinases, it is discouraging that most of the phosphorylatable residues identified, or their flanking sequences, are very poorly conserved across species, making it difficult to argue that these residues have been essential throughout evolution for the most conserved metabolic functions of SIRT1 orthologs across species. Furthermore, the metabolic roles of these phosphorylation events, as well as their interaction with other post-translational modifications, needs to be addressed in vivo to fully understand their true biological function.

A second type of post-translational modification that can affect SIRT1 activity is SUMOylation. SIRT1 is SUMOylated at Lys^{734} upon UV irradiation or H_2O_2 treatment (Yang et al., 2007c). The SUMOylation of SIRT1 increased its intrinsic deacetylase activity (Yang et al., 2007c). Conversely, mutation of the residue or forced de-SUMOylation by the small ubiquitin-like modifier (SUMO) 1/sentrin specific peptidase 1 enzyme, rendered SIRT1 less enzymatically active and cells more prone to apoptosis (Yang et al., 2007c). Although it was speculated that SIRT1 SUMOylation acts as a switch between cell survival and cell death, further work is required to define the mechanisms by which cellular stress enhances the interaction of SIRT1 with SUMOylation enzymes and/or decreases the association with SENP. As with phosphorylation, an additional caveat is the poor conservation of the SUMOylation residue and its flanking regions. For example, mouse SIRT1 cannot be SUMOylated because the human Lys^{734} is an Arg residue in mice. Although this does not rule out a potential effect of SUMOylation on SIRT1 activity in many species, including human, it is unlikely that this will contribute to the activity of SIRT1 in other species, unless SUMOylation takes place at other residues.

It is often overlooked that SIRT1 activity can affect many different targets. It is unlikely then, that, upon its activation, SIRT1 unselectively deacetylates all of its targets, which could often lead to opposite physiological effects. Therefore, some specification must exist. A clear hint that this is the case was already mentioned by the work on JNK1 (Nasrin et al., 2009), which illustrated how, despite being intrinsically more active, SIRT1 deacetylated only specific substrates. Consequently, post-translational modifications might not only affect SIRT1 activity at the level of its intrinsic activity but also channel SIRT1 toward specific subsets of targets. It is noteworthy that we have illustrated how phosphorylation of PGC-1α by AMPK is essential for SIRT1-mediated deacetylation (Cantó et al., 2009). Given the large number of SIRT1 substrates, it is

very likely that substrate accessibility is also controlled by post-translational modifications. Understanding these specification mechanisms will be essential to designing future strategies aimed to selectively affect certain functions of SIRT1 but not others.

C. Protein Interactions

SIRT1 activity is controlled not only intrinsically at the level of the SIRT1 protein but also through its association with different protein complexes, which may affect its activation or inhibition as well as its target specificity. In section II.C, we specified how the presence of SIRT1 in a complex with NCoR1 actually inhibits PPARγ action in adipose tissue. In this section, we discuss two additional and physiologically relevant mammalian SIRT1 "nonsubstrate" interactors.

Two simultaneous reports in 2007 indicated that the nuclear protein deleted in breast cancer-1 (DBC1) forms a stable complex with the catalytic domain of SIRT1 and inhibits SIRT1 activity both in vivo and in vitro (Kim et al., 2008; Zhao et al., 2008). As a consequence, the artificial reduction of DBC1 in cell-based experiments stimulated SIRT1 activity, diminishing the acetylation levels of p53 and inhibiting p53-dependent apoptosis (Kim et al., 2008; Zhao et al., 2008). The physiological pathways influencing the dynamic interaction of SIRT1 and DBC1, however, remain unexplored. The in vitro evidence indicating that DBC1 is a SIRT1 inhibitor was further supported by observations in mouse liver indicating that DBC1 and SIRT1 colocalize within the nucleus and coimmunoprecipitate in nuclear extracts (Escande et al., 2010). Mice with a germline deletion of the *DBC1* gene showed a 2- to 4-fold increase in endogenous SIRT1 activity in a wide range of tissues, rendering p53 hypoacetylated (Escande et al., 2010). The dynamics of the DBC1/SIRT1 interaction have been evaluated in vivo, demonstrating that, under normal feeding conditions, at least 50% of total liver SIRT1 is associated with DBC1, and that this interaction was nearly absent after starvation, contributing to the increase in SIRT1 activity observed in fasting livers (Escande et al., 2010). It is noteworthy that the increase in SIRT1 activity during fasting was blunted in DBC1 knockout mice (Escande et al., 2010). In contrast to the effects observed during fasting, high-fat feeding stabilized the association of DBC1 with SIRT1 (Escande et al., 2010). Understanding the molecular determinants influencing the association between DBC1 and SIRT1 will be an interesting field for future investigation. The overall phenotype of the DBC1 knockout mice did not differ from wild-type littermates on chow diet. These mice, however, were protected against the development of hepatic steatosis and liver damage induced by high-fat feeding (Escande et al., 2010), in line with the data obtained in the liver-specific SIRT1 knockout models (Purushotham et al., 2009; Wang et al., 2010).

Around the same time that DBC1 was reported as a sirtuin inhibitor, another report identified a possible activator of SIRT1 activity, the active regulator of SIRT1 (AROS) (Kim et al., 2007b). The association of AROS with SIRT1 in, presumably, its catalytic domain enhances SIRT1 activity 2-fold, resulting in p53 inhibition through deacetylation (Kim et al., 2007). Conversely, the artificial reduction in AROS levels sensitized cells to p53-induced apoptosis (Kim et al., 2007). It is relevant that AROS interacts specifically with SIRT1 but not with other sirtuins (Kim et al., 2007). The exact nature of the actions of AROS on SIRT1 and how it affects metabolism will require future study.

These data on NCoR1, DBC1, and AROS illustrate how SIRT1 activity is influenced by its association with specific protein partners. It will be important to elucidate whether these partnerships also influence SIRT1 substrate selectivity. It is reasonable to think that the known and yet-to-be identified SIRT1 interactors also have an impact on the deacetylation level of SIRT1 substrates other than p53, such as PGC-1α and FOXO1, which could have far-reaching implications for metabolic regulation. It will also be interesting for future studies to understand to what extent the deacetylase activity of SIRT1 influences the activity of corepressor or coactivator complexes and to identify the possible roles of SIRT1 as an adaptor protein. It goes without saying that modifying the interaction of SIRT1 with such interactors could constitute a promising avenue to modulate SIRT1 activity.

D. Sirtuin 1-Activating Compounds

An obvious strategy to artificially enhance SIRT1 would be through chemical compounds that directly bind and activate SIRT1. Howitz et al. (2003), using a screening strategy with a fluorescently labeled substrate, identified resveratrol and a few other polyphenols, including quercetin and piceatannol, as natural compounds that could enhance the deacetylating activity of SIRT1. A number of subsequent studies showed activated SIRT1 in diverse species (for review, see Baur, 2010). Resveratrol treatment mimics numerous aspects of calorie restriction in all eukaryotes tested to date (Howitz et al., 2003; Wood et al., 2004; Baur et al., 2006; Lagouge et al., 2006; Valenzano et al., 2006; Barger et al., 2008; Pearson et al., 2008); in most of them, this effect seems to depend on SIRT1 (Howitz et al., 2003; Wood et al., 2004; Lagouge et al., 2006). Not surprisingly, in several models tested (Howitz et al., 2003; Wood et al., 2004; Baur et al., 2006; Valenzano et al., 2006), albeit not all (Pearson et al., 2008), resveratrol increased lifespan. In mice, resveratrol promoted SIRT1 activation and energy expenditure (Baur et al., 2006; Lagouge et al., 2006). Upon high-fat feeding, resveratrol prominently prevented the onset of diet-induced obesity and metabolic disease, which ended up protecting the treated mice against the lifespan curbing associated with high caloric diets (Baur et al., 2006; Lagouge et al., 2006). At the molecular level, resveratrol boosted mitochondrial content, as a result of the activation of the SIRT1/PGC-1α axis (Lagouge et al., 2006). Resveratrol also improved mitochondrial function and fatty acid oxidation in humans, as dem-

onstrated in a recent study, but at much lower concentrations that those used in mice (Timmers et al., 2011). All together, resveratrol proved to be an effective way to activate SIRT1 in vivo and promote beneficial health effects, most of which resemble the effects observed upon the overexpression of SIRT1.

However, the ability of resveratrol to directly activate SIRT1 was seriously questioned by results demonstrating that the nonphysiological fluorescent "Fluor de Lys" substrate used for SIRT1 activity assays can lead to artifactual results (Borra et al., 2005; Kaeberlein et al., 2005). Blinded by the beneficial effects in line with SIRT1 activation, resveratrol actions on other possible molecules/pathways have been largely neglected. In fact, resveratrol was much earlier reported as a polyphenol that interfered with the mitochondrial respiratory chain (Zini et al., 1999). It is relevant, furthermore, that many reports have recently demonstrated that resveratrol can also activate AMPK (Baur et al., 2006; Zang et al., 2006; Dasgupta and Milbrandt, 2007; Park et al., 2007; Feige et al., 2008), which is consistent with its possible effect on the mitochondrial respiratory chain. Elegant studies, using isogenic cell lines stably expressing AMPK complexes containing AMP-insensitive γ2 subunit variants (R531G), convincingly demonstrated that AMPK activation in response to resveratrol derives from an AMP/ATP imbalance as a consequence of interference with mitochondrial respiration (Hawley et al., 2010). AMPK activation by resveratrol is very fast and already prominent after a few minutes, whereas the activation of SIRT1 becomes detectable only after a few hours (C. Cantó and J. Auwerx, unpublished observations), clearly indicating that AMPK activation is an earlier event upon resveratrol treatment. Although it has been argued that resveratrol action on AMPK is SIRT1-dependent (Suchankova et al., 2009), the use of mouse embryonic fibroblast cells from SIRT1 knock-out mice unequivocally demonstrated that SIRT1 is dispensable for resveratrol-induced AMPK activation (Dasgupta and Milbrandt, 2007; Um et al., 2010). Conversely, several approaches have convincingly proven that resveratrol cannot activate SIRT1 in the absence of functional AMPK (Cantó et al., 2010; Um et al., 2010). A final picture of the actions of resveratrol on AMPK and SIRT1 activation was finally drawn by the demonstration that SIRT1 is the downstream mediator of AMPK actions. AMPK activation initially leads to a gradual increase in NAD$^+$ levels, as a consequence to the activation of fatty acid oxidation, subsequently activating SIRT1 (Cantó et al., 2009). This initial raise in NAD$^+$ is sustained by enhanced Nampt expression, which favors the synthesis of NAD$^+$ from NAM through the NAD$^+$ salvage pathway (Fulco et al., 2008). The requirement for NAD$^+$ accumulation also explains why the activation of SIRT1 is not immediate upon resveratrol treatment. Therefore, SIRT1 may be essential for resveratrol action but as a downstream consequence of AMPK activation, rather than as a direct molecular target of resveratrol (Fig. 5).

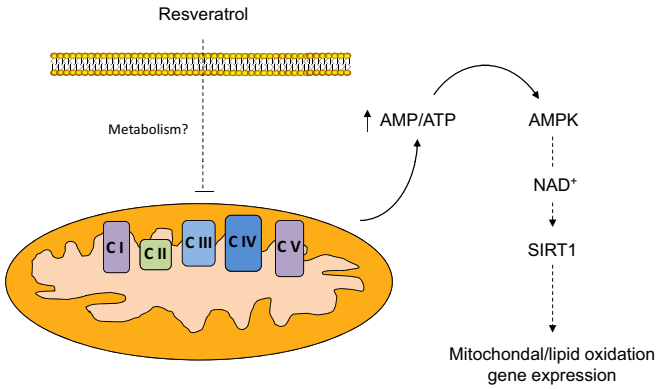


FIG. 5. Resveratrol promotes mitochondrial biogenesis and lipid oxidation gene expression through indirect AMPK and SIRT1 activation. Although still a matter of debate, most data currently indicate that the metabolic actions of resveratrol or its metabolites stem from its ability to act as a mild mitochondrial poison, impairing ATP synthesis. The energy stress induced by resveratrol activates AMPK, subsequently stimulating SIRT1 by enhancing NAD$^+$ levels. Then, SIRT1 activates key downstream targets through deacetylation (see section II.C), ultimately leading to an adaptive potentiation of mitochondrial biogenesis and lipid oxidation pathways. CI-V represent mitochondrial respiratory complexes I to V.

A more recent screening for other possible small molecular SIRT1 activators provided a second batch of compounds, among which *N*-[2-[3-(piperazin-1-ylmethyl)imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]quinoxaline-2-carboxamide (SRT1720) has been best characterized (Milne et al., 2007). SRT1720 was in vitro a much more potent and efficient activator of SIRT1 than resveratrol (Milne et al., 2007). Treatment of rodents with SRT1720 prevented diet-induced obesity and ameliorated the diabetic phenotype of genetically obese mice (Milne et al., 2007; Feige et al., 2008). Similar to what was observed with resveratrol, SRT1720 enhanced SIRT1 activity, oxidative metabolism, and mitochondrial biogenesis in mouse tissues (Feige et al., 2008). Pacholec et al. (2010) also indicated that direct activation of SIRT1 by SRT1720 observed in the in vitro setting suffered from the same artifacts derived from the use of the "Fluor de Lys" moiety and that were already described for resveratrol. Therefore, one needs to consider that SIRT1 activation in tissues from mice fed with SRT1720 might be an indirect event, potentially again involving activation of AMPK, which was evident upon long-term treatment of SRT1720 in vivo (Feige et al., 2008). In addition to that, although the in vitro assays indicated that SRT1720 was a more potent activator of SIRT1 than resveratrol, this was not translated in vivo, indicating that the actions of these compounds in vivo involve indirect activation of SIRT1 (Feige et al., 2008) and/or rather poor bioavailability.

E. Indirect Modulation through Affecting NAD$^+$ Metabolism

We have described in section II.B how sirtuins in general, and SIRT1 in particular, meet all the requirements to act as intracellular NAD$^+$ sensors. Therefore, enhancing NAD$^+$ availability could be used to activate SIRT1 and

promote beneficial health effects. We will now review different strategies used to achieve such goal and discuss whether the experimental results support that modulating NAD^+ metabolism might be a useful tool for health benefits (Fig. 6).

1. Modulation by Increasing NAD+ Synthesis. The most obvious strategy for testing how enhancing NAD^+ levels affects sirtuin activity consists in boosting NAD^+ synthesis by supplementation with NAD^+ precursors. Different precursors can be used to promote NAD^+ synthesis. The primary de novo synthesis of NAD^+ generally initiates from tryptophan (Houtkooper et al., 2010). Nicotinic acid (NA) is another NAD^+ precursor that is transformed into NAD^+ through the Preiss-Handler pathway, therefore converging with the same NAD^+ synthesis pathway used by tryptophan. It is assumed, however, that perhaps the principle source of NAD^+ comes from salvage pathways from other adenine nucleotide metabolites (Houtkooper et al., 2010). The main NAD^+ precursors that funnel through the salvage pathways are NAM and the more recently described nicotinamide riboside (NR). NAM generates NAD^+ through an independent pathway, in which the rate-limiting enzyme Nampt transforms NAM into NMN, which on its turn is converted into NAD^+ by NMN adenylyltransferase (Revollo et al., 2004). NR is phosphorylated upon its entry in the cell by the NR kinases, generating NMN, which is then converted to NAD^+ by NMN adenylyltransferase (Bieganowski and Brenner, 2004).

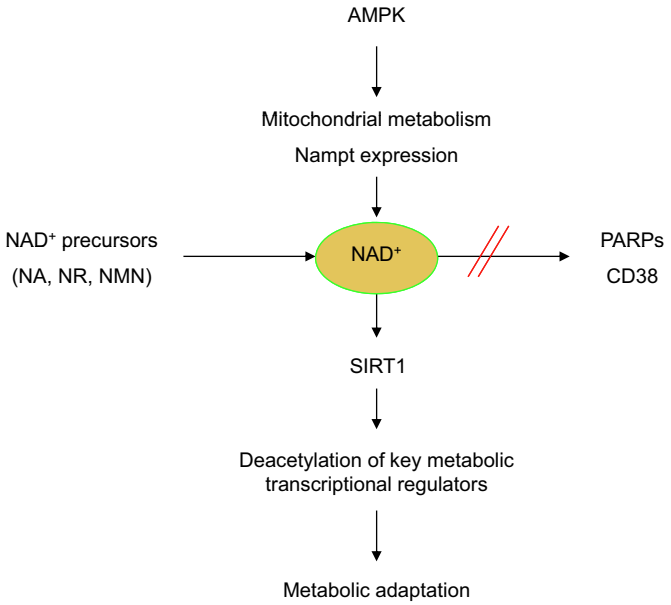


FIG. 6. NAD^+ as a nodal point for metabolic regulation. Most evidence to date points out that NAD^+ could be rate-limiting for the SIRT1 reaction in diverse conditions. SIRT1 activity would hence be stimulated by interventions that increase NAD^+ levels, such as AMPK activation, enhancement of NAD^+ biosynthesis through precursor (NA, NR, NMN) supplementation, or through inhibition of alternative NAD^+ consuming activities, such as PARPs or CD38. The stimulation of SIRT1 activity by these distinct means improves the capacity of the cells/organism to adapt to external metabolic cues.

Few studies to date have described how supplementation with these precursors influence NAD^+ levels and in particular affect sirtuin activity. Both NA and NAM can lead to higher NAD^+ levels, even though the effects might be tissue-specific. NAM seems to be a more stable NAD^+ precursor in the liver (Collins and Chaykin, 1972), but NA seems to be a more efficient in the kidney (Hara et al., 2007). The metabolism of these NAD^+ precursors by the gut flora and intestinal enzymes can also contribute to their absorption and their efficacy to increase NAD^+ (Gross and Henderson, 1983). Cell-based experiments also support the existence of cell-specific differences. For example, in NIH3T3 cells, concentrations of up to 5 mM NAM were unable to increase intracellular NAD^+ levels (Revollo et al., 2004). Although NAM was also a relatively inefficient NAD^+ precursor in human embryonic kidney 293 cells, requiring 5 mM concentrations to increase NAD^+, NA was more efficient, and concentrations as low as 20 μM doubled the NAD^+ content (Hara et al., 2007). The cell/tissue-specific efficiencies of distinct NAD^+ precursors might be consequent to the differential expression of the rate-limiting enzymes in their respective metabolic pathways. NA metabolism into NAD^+ is rate-limited by the NA phosphoribosyltransferase, which is highly enriched in some tissues, such as liver or kidney (Hara et al., 2007). A reduction in NA phosphoribosyltransferase activity in human embryonic kidney 293 cells does not affect basal NAD^+ levels but impairs NA-induced NAD^+ accumulation (Hara et al., 2007). To date, however, no clear link between NA supplementation and sirtuin activity has been made.

Both NA and NAM have been used for a long time in a clinical setting. Niacin, basically composed of NA and NAM, has been widely used as an efficient way to overcome situations of dietary tryptophan deficits (Sauve, 2008). Niacin is also used to treat hypercholesterolemia, because it efficiently decreases very-low-density lipoprotein synthesis, lowers low-density lipoprotein cholesterol levels, and concurrently increases high-density lipoprotein cholesterol (Altschul et al., 1955; Karpe and Frayn, 2004). The use of niacin as a lipid-lowering agent results in beneficial effects on coronary artery disease and type 2 diabetes mellitus. It is not clear whether these effects rely on SIRT1 activation. Many of the beneficial actions of niacin in mice and humans—as well as some of the undesired effects, such as spontaneous flushing—have been attributed to the activation of a putative NA-activated G-protein-coupled receptor, GPR109A (Tunaru et al., 2003; Benyó et al., 2005). Whereas the role of GPR109A in mediating niacin-induced flushing is rather well established (Benyó et al., 2005), the hypothesis that the therapeutic efficacy of niacin is mediated by GPR109A activation needs to be revisited. First, even if GPR109A has a relatively high affinity for NA (EC_{50}, ~100 nM), such levels of NA are rarely found in plasma unless pharmacologically primed (Kirkland, 2009), indicating that the activation of GRP109A by NA is probably fortuitous but not biologically relevant in the basal state. Second, many of the

beneficial effects of niacin, such as the lipid-lowering effects (Kamanna and Kashyap, 2008), take place at concentrations higher than those required for GPR109A activation but lead to intracellular NAD$^+$ accumulation (Jackson et al., 1995). It is therefore tempting to speculate that some of the effects promoted by niacin might be achieved through an NAD$^+$-induced activation of SIRT1 and the consequent deacetylation of the multiple SIRT1 targets that act as critical regulators of fatty acid, lipid and sterol homeostasis in eukaryotes, such as PGC-1α, FOXOs, LXR, or SREBP-1c (described in section II.C). An additional appealing possibility to explain the effects of niacin involves the fact that it leads to adiponectin release from the white adipose tissue (Vaccari et al., 2007; Westphal et al., 2007). Adiponectin then activates AMPK in muscle and liver tissues, which would enhance NAD$^+$ content and SIRT1 activity (Cantó et al., 2009; Iwabu et al., 2010). Therefore, future work is urgently needed to elucidate the contribution of SIRT1 as a potential mediator of niacin's beneficial health effects.

The effects of NR, the most recently discovered NAD$^+$ precursor, remain also largely unknown. Although NR can increase intracellular NAD$^+$ in mammalian cells (Yang et al., 2007b), it is still not reported whether this is enough to influence sirtuin activity. However, evidence obtained in yeast models suggests that this might in fact be the case. Supplementation of yeast with NR enhanced Sir2-dependent repression of recombination, improved gene silencing and extended replicative lifespan (Belenky et al., 2007). All these actions were completely dependent of NAD$^+$ synthesis (Belenky et al., 2007). These experiments using NR constitute the first solid evidence that enhancing NAD$^+$ bioavailability via NAD$^+$ precursor supplementation can also enhance sirtuin activity and increase lifespan, even though they are limited to yeast models. Furthermore, the discovery that NR is present in cow's milk (Bieganowski and Brenner, 2004) poses an interesting opportunity for food-based preventive or therapeutic interventions in NAD$^+$-dependent metabolism.

In addition to boosting NAD$^+$ levels through administration of NAD$^+$ precursors, NAD$^+$ levels can also be artificially modulated by changing the activity of rate-limiting enzymes in NAD$^+$ biosynthesis. This principle is well illustrated by the modulation of Nampt activity, which has a clear impact on NAD$^+$ levels in virtually any mammalian cell tested (for examples, see van der Veer et al., 2005; Fulco et al., 2008; Rongvaux et al., 2008; Pittelli et al., 2010). In most of these cases, the alterations in NAD$^+$ levels promoted by differential Nampt activity were associated with changes in SIRT1 activity (van der Veer et al., 2005, 2007; Revollo et al., 2007; Fulco et al., 2008). However, and as explained in section II.B, a complication from these experiments is that the influence that Nampt exerts on SIRT1 activity may derive not only from altering NAD$^+$ availability but also from more effective NAM clearance (Bitterman et al., 2002). Nampt inhibitors are currently being developed to deplete NAD$^+$ levels and as such induce tumor cell apoptosis (Hasmann and Schemainda, 2003). In addition, inhibitors of kynurenine 3-monooxygenase, involved in de novo NAD$^+$ biosynthesis pathways, have already been identified (Zwilling et al., 2011). Despite the facility to develop enzyme inhibitors, it will be more challenging to discover and develop compounds that pharmacologically activate the NAD$^+$ salvage and/or de novo synthesis pathways. Such activators could potentially enhance NAD$^+$ levels and activate SIRT1 to promote metabolic fitness.

2. Modulation by Decreasing NAD$^+$ Consumption. Another attractive way to modulate NAD$^+$ levels and favor sirtuin activity resides in the modulation of the activity of nonsirtuin NAD$^+$-consuming enzymes. There are two major families of alternative NAD$^+$ consumers: the PARPs and the cADP-ribose synthases (CD38 and CD157) (Houtkooper et al., 2010) (Fig. 6).

a. Poly(ADP-ribose) Polymerase. PARPs use NAD$^+$ as a substrate for a cellular process in which the ADP-ribose moiety is not transferred to an acetyl group, as happens with sirtuins, but is used to build ADP-ribosyl polymers onto acceptor proteins (Chambon et al., 1963; Krishnakumar and Kraus, 2010). PARP activity is robustly enhanced upon DNA damage and oxidative stress. Most PARP activity upon oxidative damage is driven by PARP-1, except for a residual 5 to 10%, which is accounted by PARP-2 (Shieh et al., 1998; Amé et al., 1999). Overactivation of PARP-1 upon oxidative damage rapidly depletes intracellular NAD$^+$ levels (Goodwin et al., 1978; Skidmore et al., 1979). In line with the hypothesis that NAD$^+$ might be rate-limiting for SIRT1 action, SIRT1 activity is down-regulated in situations of PARP-1 activation (Pillai et al., 2005; Bai et al., 2011b). Significantly, SIRT1 does not seem to be directly regulated through poly-ADP-ribosylation events (Bai et al., 2011a,b), indicating that PARP-1 and SIRT1 activity are connected through competition for a limited NAD$^+$ pool. PARP-1 has a very low K_m (~20 μM) and a relatively high V_{max} for NAD$^+$ (Mendoza-Alvarez and Alvarez-Gonzalez, 1993), indicating that PARP-1 might limit SIRT1 action but not the other way around.

Bai et al. (2011b) have demonstrated how down-regulation of PARP-1 activity favors SIRT1 activation. Genetic and pharmacological approaches that abrogate PARP-1 activity induced NAD$^+$ levels. Although PARP activity is generally accepted to be rather low in the basal state, recent evidence indicates that it naturally fluctuates in a circadian fashion (Asher et al., 2010). In line with the existence of basal PARP activity, inhibition of its activity gradually leads to a build up of NAD$^+$ levels in cultured cells (Bai et al., 2011b). Likewise, tissues from PARP-1 knockout mice have an increased NAD$^+$ content (Bai et al., 2011b), roughly 2-fold higher than wild-type littermates. The increase in NAD$^+$ levels promoted by PARP inhibition leads, both in vivo and in vitro, to higher SIRT1 activity (Bai et al., 2011b). This way, PARP inhibition associates with the induction of the expression of genes involved in

mitochondrial and lipid oxidation in a SIRT1-dependent manner. From a physiological perspective, the better metabolic fitness derived from SIRT1 activation offers the PARP-1-null mice protection against the onset of metabolic disease in the context of diet-induced obesity (Bai et al., 2011b). More acute inhibition of PARP activity, using pharmacological PARP inhibitors, was also enough to enhance mitochondrial gene expression in vivo and in vitro (Bai et al., 2011b). There are currently nine different PARP inhibitors at different stages of clinical development and at least three highly selective PARP inhibitors in late preclinical development against diverse types of cancer (for review, see Yuan et al., 2011b). It is noteworthy that most cancer cells rely on anaerobic glycolysis to sustain their high proliferation rates, even if oxygen is plentiful, an event that is known as "the Warburg effect" (after Otto Warburg, who first described it) and is believed to be key for malignant transformation. Apart from their undisputed effect on DNA repair (Krishnakumar and Kraus, 2010), PARP inhibitors might also contribute to inhibit cancer progression by promoting oxidative metabolism and acting as anti-Warburg agents. Because most PARP inhibitors target both PARP-1 and PARP-2, they would furthermore potentiate SIRT1 function both at the enzymatic level—through PARP-1 inhibition and raising NAD^+ levels—and at the transcriptional level—through PARP-2 inhibition and induction of SIRT1 expression (see section IV.A). Furthermore, the results mentioned above also indicate that PARP inhibitors may be retooled to boost oxidative metabolism in metabolic diseases in which mitochondrial function is impaired, such as inherited and acquired mitochondrial diseases.

The interaction between PARP-1 and SIRT1 activities also opens the perspective of a possible role of PARPs in aging, because SIRT1 activity has been postulated as a mediator of the beneficial effects of calorie restriction on health and lifespan (Cantó and Auwerx, 2011). Hence, PARP inhibition could be a nice strategy to activate SIRT1 and mimic the calorie-restricted state. In line with this hypothesis, it has been reported that PARP activity is higher in aged tissues, leading to decreased SIRT1 activity (Braidy et al., 2011; C. Cantó, R, Houtkooper, L. Mouchiroud, and J. Auwerx, unpublished observations). Confirming the hypothesis that higher PARP-1 activity might be detrimental for SIRT1 function and global metabolism was the fact that mice expressing an additional copy of the human PARP-1 have reduced median lifespan, impaired glucose homeostasis, and higher susceptibility to age-related diseases (Mangerich et al., 2010). Further studies should evaluate the possible influence of PARP activity on aging.

It is of note that the NAD^+ boosting effects of PARP inhibition enhance the activity of SIRT1, but not that of SIRT2 or SIRT3 (Bai et al., 2011b). The major difference between these three sirtuins is their subcellular localization, because, among them, only SIRT1 is a nuclear sirtuin. This suggests the existence of compartment-specific NAD^+ pools in the cell. Supporting this possibility, elegant studies by Yang et al. (2007a) showed the existence of independently regulated NAD^+ pools.

b. CD38. Another small family of NAD^+ consumers are the cADP-ribose synthases, CD38 and the less characterized CD157. CD38 and CD157 are multifunctional enzymes that use NAD^+ as substrate to generate second messengers, mainly cADP-ribose, which, in turn, regulates calcium mobilization (Lee, 2006). Most studies on how cADP-ribose synthases affect NAD^+ levels and SIRT1 activity have been done with CD38. As is true for PARP-1, CD38 displays a very low K_m for NAD^+ (15–25 μM) (Cakir-Kiefer et al., 2001). The stoichiometry of the reaction catalyzed by CD38 involves massive amounts of NAD^+, around 100 molecules, to yield a single cADP-ribose (Dousa et al., 1996). Therefore, even low levels of CD38 activity might have a dramatic influence on intracellular NAD^+ metabolism. Remarkably, the enzymatic activity of CD38 is located outside the plasma membrane (De Flora et al., 1997), which makes it challenging to envision how NAD^+ can access this enzyme, because NAD^+ is only present in minute quantities outside the cell (O'Reilly and Niven, 2003). It was recently suggested, however, that CD38 might also be present in the nuclear compartment, which would then set the stage for a role of CD38 as a main intracellular NAD^+ consumer (Aksoy et al., 2006). The function of CD38 as an intracellular NADase was subsequently proven right when mice lacking CD38 displayed a 30-fold increase in intracellular NAD^+ levels (Aksoy et al., 2006b). This increase in NAD^+ levels is far superior compared with the ~2-fold increases generally observed in most genetic (PARP-1 deletion), pharmacological (NAD^+ precursors), or physiological interventions (fasting, calorie restriction) that enhance NAD^+ content. The increase in intracellular NAD^+ elicited by CD38 deletion significantly activated SIRT1 and prompted clinical phenotypes similar to those expected for SIRT1 activation, including protection against diet-induced obesity and a robust deacetylation of SIRT1 targets (Aksoy et al., 2006). The fact that the 30-fold increase in NAD^+ triggered by *CD38* gene deletion has a biological effect similar to the 2-fold effect observed in PARP-1 knockout mice suggests that the effective NAD^+-sensing range of SIRT1 might be largely exceeded in the CD38 knockout mice. Given the nuclear and plasma membrane localization of CD38, it will be interesting to evaluate whether the increase in NAD^+ is homogeneous between different cellular compartments and whether other sirtuins are similarly affected. As for the PARPs, specific CD38 inhibitors are being developed (Sauve and Schramm, 2002; Dong et al., 2011), and it will be relevant to evaluate how they influence SIRT1 activity in situations where oxidative metabolism is impaired (e.g., acquired and inherited mitochondrial defects).

V. Conclusions and Future Directions

The wealth of new data on SIRT1, generated in species beyond yeast, has recently spontaneously refocused the attention of the field from a potential—but so far not clearly proven—role in increasing lifespan toward its ability to modulate whole-body metabolism. Probably evolved as a mediator of the metabolic and transcriptional adaptations to situations of energy stress and nutrient deprivation, SIRT1 activation enhances the ability of organisms to enhance fat consumption and use mitochondrial respiration as a way optimize energy harvesting. Metabolic disease has been strongly linked to impaired energy homeostasis and mitochondrial function. Therefore, manipulations aimed to enhance SIRT1 activity might turn out to be attractive for the prevention and treatment of metabolic disease. Studies in genetic engineered mouse models support the notion that higher SIRT1 activity is protective against metabolic disease without necessarily influencing lifespan. In contrast, defective SIRT1 activity in mouse models is generally linked to metabolic inefficiency. Although the quest for direct SIRT1 activators has delivered interesting compounds, such as resveratrol and SRT1720, most of them have been proven to activate SIRT1 in very indirect and unspecific fashion in vivo. Therefore, novel approaches to activate SIRT1 have emerged. Among them, strategies aimed to modify the intracellular NAD$^+$ content have provided exquisite correlative evidence that higher NAD$^+$ bioavailability is matched by higher SIRT1 activity. It is noteworthy that the activation of SIRT1 seems to be a key convergent downstream effect, common between the many different approaches used to modulate NAD$^+$ levels, such as increasing NAD$^+$ synthesis rates or decreasing NAD$^+$ consumption rates. This way, the modulation of NAD$^+$ levels appears as one of the most promising strategies to control SIRT1 activity to achieve health benefits. One of the advantages of raising NAD$^+$ levels is that that it can be achieved from many different angles and strategies that boost NAD$^+$ levels or inhibit NAD$^+$ consumption are being pursued for nutraceutical (e.g., NA precursors) and pharmaceutical (e.g., PARP and CD38 inhibitors) development. It should furthermore not be forgotten that despite the fact that raising NAD$^+$ levels might allow SIRT1 to reach higher enzymatic rates, other factors, ranging from transcriptional and translational to post-translational (e.g., post-translational modifications in the narrow sense and protein complex formation) mechanisms, also profoundly affect SIRT1 activity. In our review, we have highlighted how different post-translational modifications in SIRT1 or in its substrates can strongly drive SIRT1 activity toward specific cellular processes. This indicates that the activity of sirtuins on certain substrates might be coupled not only to NAD$^+$ availability but also to the accessibility of the target, which is determined by another post-translational modification. This case is perfectly illustrated by PGC-1α, the deacetylation of which is much more efficient when phosphorylated. Likewise, it will be very relevant to understand how the ability of SIRT1 to interact with specific substrates is determined by the cohabitation with other molecular coactivator (e.g., PGC-1α) or corepressor (e.g., NCoR1) complexes. The complexity of SIRT1 physiology, which is largely conserved throughout evolution, makes unraveling the intricacy of the SIRT1 signaling and putting it to fruition in drug development a challenging task that will occupy scientists for years to come. Therefore, those who are waiting for a SIRT1-activating entity that will grace them with eternal youth will have to wait a little longer.

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Wrote or contributed to the writing of the manuscript: Cantó and Auwerx.

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