Sirtuins, Aging, and Metabolism

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Sirtuins are nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases that link protein acetylation, metabolism, aging, and diseases of aging. Sirtuins were initially found to slow aging in lower organisms and more recently shown to mediate many effects of calorie restriction on metabolism and longevity in mammals. This chapter focuses on two key mammalian sirtuins, SIRT1 (which resides mainly in the nucleus) and SIRT3 (which is mitochondrial). I discuss the many protein substrates of these sirtuins and how they determine the metabolic strategy most efficacious under scarce or abundant food supplies. I also discuss the logic by which sirtuins link protein acetylation and metabolism. Finally, I discuss emerging data showing protection by sirtuins against most of the common diseases of aging. It is possible that sirtuins will be novel targets to combat these diseases pharmacologically.

Sirtuins are NAD-dependent protein deacetylases that are homologous to yeast *Sir2p*, one of the genes involved in silencing in that organism (Klar et al. 1979). Interest in sirtuins grew when *Sir2p* was shown to slow aging in yeast mother cells (Kaeberlein et al. 1999). Subsequent studies showed similar effects on aging in *Caenorhabditis elegans* and *Drosophila* (Tissenbaum and Guarente 2001; Rogina and Helfand 2004; Viswanathan et al. 2005; Berdichevsky et al. 2006; Bauer et al. 2009; Rizki et al. 2011; Viswanathan and Guarente 2011). Interest in sirtuins grew further when the yeast *Sir2p* and mammalian ortholog SIRT1 were shown to be NAD-dependent deacetylases (or class III deacetylases; Imai et al. 2000). In this unique reaction, NAD itself is cleaved every deacetylation cycle to generate *O*-acetyl ADP-ribose and nicotinamide, which are subsequently reassembled into NAD (Tanner et al. 2000).

Mammals have seven sirtuins, which are functionally nonredundant. SIRT1, SIRT6, and SIRT7 are primarily nuclear proteins, SIRT3, SIRT4, and SIRT5 are imported into mitochondria, and SIRT2 is cytoplasmic (Finkel et al. 2009; Haigis and Sinclair 2010; Verdin et al. 2010; Guarente 2011). Sirtuins execute their function by deacetylating many target proteins in the different cellular compartments. The physiological effects of deacetylation of sirtuin targets may be classified into two categories—the control of metabolism and the response to oxidative stress. In the first category, SIRT1 deacetylates many transcription factors that control the choice by cells of an oxidative versus glycolytic metabolic strategy, among them PGC-1 α , HIF-1 α , and PPAR α (and other nuclear receptors; Rodgers et al. 2005; Purushotham et al. 2009). In addition, sirtuins deacetylate numerous metabolic enzymes to govern their specific activity. For example, SIRT1 and SIRT3 deacetylate the nuclear and mitochondrial isoforms of

acetyl coenzyme-A (Co-A) synthetase to activate it by removing an acetyl group that blocks the active site (Hallows et al. 2006; Schwer et al. 2006). The sum of all of the known activities of sirtuins on transcription factors and metabolic enzymes would program cells for oxidative metabolism in mitochondria.

Sirtuins also help mediate the response to stress. SIRT1 deacetylates heat shock factor (HSF) to increase the perdurance of the heat shock response (Westerheide et al. 2009). Oxidative stress generates DNA damage, and SIRT1 and SIRT6 deacetylate numerous components of the DNA repair machinery, such as Ku70 and PARP (Haigis and Sinclair 2010; Nakagawa and Guarente 2011). Transcriptional targets of SIRT1, such as p53 and FOXO, also govern the cellular timing that balances repair versus apoptosis during the damage response. In addition, SIRT3 plays a major role in suppressing reactive oxygen species (ROS) in mitochondria, the primary source of oxidative stress, by deacetylating antioxidant enzymes and components of the electron transport chain itself (Bell and Guarente 2011). Finally, SIRT1 and SIRT6 both suppress another manifestation of ROS, the proinflammatory response, by suppressing the activity of the key regulator of inflammation, NF- κ B. SIRT1 deacetylates p65 of NF- κ B to suppress its ability to activate proinflammatory transcriptional targets (Yeung et al. 2004), and SIRT6 deacetylates histones at the promoters of NF- κ B-regulated genes to further suppress the proinflammatory output (Kawahara et al. 2009).

SIRTUINS LINK PROTEIN ACETYLATION AND METABOLISM

A large number of cellular proteins are acetylated. For example, recent studies have demonstrated that a high fraction of mitochondrial proteins are acetylated (Kim

et al. 2006) as well as most metabolic enzymes in the cytosolic/nuclear pool (Wang et al. 2010; Zhao et al. 2010). Acetylation in the cytosol is carried out by histone acetyltransferases (HATs) and deacetylation by class I and II histone deacetylases (HDACs) as well as sirtuins. Among the sirtuins, SIRT1 appears to have the broadest range of substrates and affect the greatest number of physiological pathways (Finkel et al. 2009; Haigis and Sinclair 2010; Imai and Guarente 2010). In the mitochondria, the source of protein acetylation is not known (and may not even be enzymatic), but the most prominent deacetylase appears to be the sirtuin SIRT3 (Lombard et al. 2007).

Cells have two metabolic strategies to produce ATP, glycolysis, which takes place in the cytosolic/nuclear location, and oxidative phosphorylation, which occurs in the mitochondria. When energy is abundant in the diet, growth factors drive glucose uptake and activation of glycolysis. Thus, ATP is generated and NAD is converted to NADH (reduced NAD) in the cytosolic/nuclear pool (Fig. 1A). Because the sirtuins are NAD-dependent deacetylases, a high flux through glycolysis will lower NAD levels and thus repress the activity of SIRT1 as well as the other sirtuins in the nucleus and cytoplasm. Indeed, many studies have shown that SIRT1 activity is low in growth conditions of glucose excess and high during energy limitation (Finkel et al. 2009; Haigis and Sinclair 2010; Imai and Guarente 2010). Low SIRT1 activity would increase the acetylation status of many metabolic enzymes in the cytosolic/nuclear compartment, as long as the activities of HATs and HDACs were constant under changing energy supplies.

Indeed, in *Salmonella*, the acetylation of many glycolytic enzymes was shown to be higher in glucose-grown cells than in cells grown on a nonfermentable carbon source (Wang et al. 2010). This difference was at least partly mediated by the bacterial sirtuin cobB, because acetylation of these enzymes appeared to be higher in cobB mutants. One enzyme regulated in this way is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate in glycolysis and catalyzes the reverse reaction in gluconeogenesis (a rare example in which the same enzyme functions at the same step of glycolysis and gluconeogenesis). Remarkably, acetylated GAPDH is better at driving the forward reaction used in glycolysis, whereas deacetylated GAPDH is more effective at the reverse reaction used in gluconeogenesis.

These simple findings suggest an autocatalytic, feed-forward mechanism—diets rich in carbohydrate energy drive glycolysis, convert NAD to NADH, inactivate sirtuins, and increase acetylation and activity of GAPDH and perhaps other glycolytic enzymes (Fig. 1A). Intriguingly, mammalian GAPDH is also acetylated, and it will be important to determine whether it, as well as other acetylated metabolic enzymes in mammalian cells, is a SIRT1 substrate.

It is informative to follow the flow of carbon processed in glycolysis. The pyruvate produced by glycolysis has two possible fates. First, it can be reduced to fermentation products (bacteria), ethanol (yeast), or lactate (mammals), thereby converting some of the NADH produced in glycolysis back to NAD. Second, it can be used to

Figure 1. Linking protein acetylation, metabolism, and sirtuins. (A) ATP production by glycolysis and flow of carbon (black text and arrows) in energy storage tissues when energy is in excess. Mitochondria (Mito) and the tricarboxylic acid (TCA) cycle are indicated, and the remainder of the cell represents the cytosolic and nuclear compartments combined. Enzymes in blue italics are glyceraldehyde-3-phosphate dehydrogenase (GAPDH), histone acetyltransferases (HATs), pyruvate dehydrogenase (PDH), and ATP-citrate lyase (ACL). Note a feed-forward loop of high glycolysis and NADH production, low SIRT1 activity, and high acetylation and activity of glycolytic enzymes (e.g., GAPDH). Carbon flows as follows. Glucose 6-phosphate (Glu-6P) is converted to pyruvate, which can be reduced to lactate or converted to acetyl-CoA (Ac-CoA) by PDH. In this case, citrate from the TCA cycle is transported to the cytosol and converted to Ac-CoA for fatty acid synthesis by ACL. Depletion of citrate from the TCA cycle necessitates anapleurotic conversion of amino acids (AA) to α -ketoglutarate to replenish the cycle. (B) ATP production by oxidative metabolism and flow of carbon (black text and arrows) in energy-producing tissues upon energy deprivation. Glycolytic feed-forward loop in A is reversed, thereby activating SIRT1 and deacetylating and inhibiting glycolytic enzymes. SIRT1 generates O-acetyl ADP ribose and thus acetate (Ac) by deacetylating glycolytic proteins and induces mitochondrial biogenesis and β -oxidation of fatty acids by deacetylating transcription factors (TFs) such as PGC-1 α . SIRT3 is also induced under energy limitation. Note that deacetylation of ACS and long-chain acyl dehydrogenase (LCAD) by SIRT3 in the mitochondria is known to activate those enzymes to generate acetyl-CoA.

synthesize fatty acids as a primary mechanism of cell growth and energy storage. In this case, the pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase (PDH) in mitochondria and then re-derived back into cytoplasmic acetyl-CoA (Fig. 1A). This step occurs by transport of citrate from mitochondria to the cytosol, where ATP-citrate lyase (ACL) converts it to acetyl-CoA. ACL was recently shown to be required for normal histone acetylation in mammalian cells (Wellen et al. 2009), suggesting that the primary pathway of storing energy as fat may also provide the carbon for protein acetylation in the cytosolic/nuclear pool. By analogy with fatty acid synthesis, the high degree of protein acetylation in the cytosolic/nuclear pool during energy excess can be seen as another mechanism of storing carbon energy in dietary-sufficient conditions.

A reverse set of considerations applies to diets poor in energy: low glycolysis, activated SIRT1, and protein deacetylation in the cytosolic/nuclear pool (Fig. 1B). These conditions would perfectly match one of the main outputs of SIRT1 activity, the deacetylation of PGC-1 α to promote mitochondrial biogenesis and oxidative metabolism (Rodgers et al. 2005). Oxidative phosphorylation produces much more ATP per input glucose than fermentation, because the fuel is oxidized all the way to CO₂. Because fatty acids are oxidized in mitochondria under these conditions, acetyl-CoA (and FADH) will be generated in this compartment. At the same time, the generation of acetyl-CoA in the cytoplasm by ATP-citrate lyase will be limited. Both the high SIRT1 activity and low cytosolic acetyl-CoA levels would favor maintenance of cytosolic/nuclear proteins in a relatively deacetylated state during energy-poor diets.

These considerations do not apply in any obvious way to mitochondrial protein acetylation. Indeed, changes in the acetylation of mitochondrial proteins with diet vary from tissue to tissue and are likely to be distinct from acetylation of proteins in the nuclear/cytoplasmic pool (Schwer et al. 2009). There are likely to be competing processes affecting protein acetylation in this organelle. On the one hand, oxidative metabolism in mitochondria, for example, fatty acid oxidation, may be expected to increase acetyl-CoA levels in the organelle and thereby favor protein acetylation, perhaps occurring chemically (Fig. 1B). On the other, one of the mitochondrial proteins activated under these conditions is the major mitochondrial deacetylase SIRT3. Two key outputs of deacetylation by SIRT3 are to activate mitochondrial metabolic enzymes for oxidative metabolism and enzymes for detoxification of ROS (Verdin et al. 2010; Bell and Guarente 2011).

Protein acetylation serves as a regulatory mechanism, as well as an energy storage mechanism, at least in the nuclear/cytoplasmic compartment. The regulatory aspects minimally include histone acetylation to regulate gene transcription, acetylation of metabolic enzymes to favor glycolysis for ATP production, and acetylation of many transcription factors, such as p53, FOXO, PGC-1 α , nuclear receptors, etc., to regulate expression levels of pathways for oxidative versus glycolytic metabolism

(Finkel et al. 2009; Haigis and Sinclair 2010; Imai and Guarente 2010). It is worth noting that the acetylation of lysines that are SIRT1 substrates can either be activating (e.g., p53) or repressing (e.g., PGC-1 α), which has evidently allowed evolution greater flexibility in tinkering with the many affected pathways.

The idea that protein acetylation is a storage mechanism is most easily appreciated in cells transitioning from energy excess to energy limitation (Fig. 1B). Under these conditions, acetate generated by SIRT1 deacetylation of many proteins would be a substrate for mitochondrial ACS. Along with the oxidation of fatty acids, this would drive the TCA cycle and oxidative phosphorylation to yield ATP and CO₂. It is rather striking that SIRT3, which is up-regulated during this energy transition, deacetylates and activates both the mitochondrial ACS and long-chain acyl dehydrogenase (LCAD) in the fatty acid oxidation pathway.

In the above model, protein acetylation governs how cells navigate between glycolytic and oxidative metabolism as a function of available energy. Because the substrates for protein acetylation are NAD and acetyl-CoA, it is not surprising that SIRT1, an NAD-dependent deacetylase, would be a central metabolic sensor and mediator of this process. The protein acetylation cycle depicted in the figure is interdigitated with other biological processes that affect the metabolic strategy of cells. For example, PARP-1 inhibition drove SIRT1 activity and oxidative metabolism by raising NAD levels (Bai et al. 2011). In addition, mice knocked out for the plasma membrane citrate transporter, INDY, showed metabolic effects similar to SIRT1 gain-of-function animals (Birkenfield et al. 2011). It will be very interesting to see whether these mice show decreased cytosolic acetyl-CoA (owing to decreased citrate in the cytosol), decreased protein acetylation, and decreased glycolysis—a pattern that is predicted to up-regulate SIRT1, as discussed in the model above.

SIRTUINS MEDIATE CALORIE RESTRICTION

Sir2p was shown to mediate extension of life span by modest glucose reduction in yeast. These findings were subsequently challenged in studies using a protocol with still lower glucose concentration in the media. In *C. elegans* and *Drosophila*, studies using different protocols have produced varying results that show dependence on sirtuins in some cases but not in others. In mice, calorie restriction (CR) is much better defined than in the lower organisms—60%–70% of ad libitum feeding. Here, many lines of evidence point to the critical nature of sirtuins in CR.

1. Sirtuin targets, including those mentioned above, are critical in the adaptation from energy excess (ad libitum feeding) to CR. This transition triggers an up-regulation of oxidative metabolism. For example, SIRT1 and SIRT3 were shown to activate genes encoding fatty acid oxidation by targeting PPAR α in the nucleus (SIRT1; Purushotham et al. 2009) and metabolic

enzymes themselves in the fatty acid degradation pathway in the mitochondria (SIRT3; Hirschey et al. 2010). SIRT3, SIRT4, and SIRT5 were also shown to regulate other mitochondrial enzymes critical for the metabolic adaptation to energy limitation, such as glutamate dehydrogenase (Haigis et al. 2006) and enzymes of the urea cycle (Nakagawa et al. 2009; Hallows et al. 2011).

2. Levels of both SIRT1 and SIRT3 have been shown to rise in several tissues during CR (Cohen et al. 2004; Haigis and Sinclair 2010). This increase may, at least in part, be due to an increase in available NAD in the nuclear pool, concomitant to a lowering in the flux through glycolysis (which reduces NAD to NADH). In muscle and white adipose tissues, this increase has been directly demonstrated (Chen et al. 2008). It was recently shown that SIRT1 activates its own expression (Xiong et al. 2011), and it has been suggested that SIRT1 may also activate SIRT3 expression (Bell and Guarente 2011). Thus, an increase in NAD could elevate the protein levels of both of these key sirtuins.
3. Loss-of-function genetic evidence shows the importance of sirtuins in CR. Deletion of SIRT1 prevented outputs of CR, such as increased physical activity and extension in life span (Chen et al. 2005; Boily et al. 2008). Brain-specific SIRT1 deletion abolished the normal regulation of the somatotropic axis by diet, which emanates from the hypothalamus (Cohen et al. 2009). Deletion of any of the mitochondrial sirtuins resulted in metabolic defects in calorie limitation, such as increased blood ammonia (Nakagawa et al. 2009). Strikingly, deletion of SIRT3 has been shown to abolish the strong protective effect of CR against hearing loss in normal aging mice (Someya et al. 2010). This last example ties together major theses in aging research: oxidative damage, CR, sirtuins, and mitochondria.
4. Gain-of-function genetic evidence also implicates SIRT1 in CR. SIRT1 transgenic mice showed protection against metabolic disease with normal aging, mirroring effects of CR on wild-type animals (Bordone et al. 2007; Banks et al. 2008; Pfluger et al. 2008; Herranz et al. 2010). Importantly, these mice were also protected against diabetes, liver steatosis, bone loss, and general inflammation caused by obesity. Deacetylation of SIRT1 targets is evidently required for these effects, because a SIRT1 transgene with a catalytic mutation did not confer benefits.
5. Small-molecule SIRT1 activators have similar metabolic benefits to the SIRT1 transgenes (Baur et al. 2006; Lagogue et al. 2006). These activators include the natural product resveratrol (Howitz et al. 2003) and newer more potent synthetic compounds (Milne et al. 2007). In the latter case, efficacy was also demonstrated in a genetic model of murine obesity (ob/ob mice; leptin deficient) and in obese rats. Some have argued that these compounds do not target SIRT1 (Pacholec et al. 2010), but the most recent report

showed direct activation of this sirtuin by the newer chemicals *in vitro* (Dai et al. 2010). Consistent with the idea that the compounds may activate sirtuins and thus function as CR mimetics, transcriptional profiling showed a significant overlap between mice on CR and mice dosed with putative SIRT1-activating compounds (Barger et al. 2008). Finally, at least some of the effects of these compounds were abolished in mice in which SIRT1 had been deleted (Boily et al. 2009; Minor et al. 2011). Given the interest in this area, these compounds are now being studied intensely and their mechanism(s) of action should be worked out soon.

SIRTIINS SLOW AGING AND DISEASES

SIRT1 and Metabolic Diseases

An early indication of the potential importance of SIRT1 in metabolic diseases was the finding that the protein could influence differentiation and fat accumulation in 3T3-L1 cells and primary rat pre-adipocytes (Picard et al. 2004). In addition, SIRT1 was shown to deacetylate and activate peroxisome proliferator-activated receptor- γ coactivator PGC-1 α (Rodgers et al. 2005), and CR triggered a SIRT1/PGC-1 α -dependent increase in muscle mitochondrial biogenesis (Nisoli et al. 2005). Limiting calories also caused SIRT1 to deacetylate and activate peroxisome proliferator-activated receptor PPAR α to turn on genes for fatty acid oxidation (Purushotham et al. 2009). In toto, these effects would favor oxidative metabolism in muscle, adipose tissues, and liver, insulin sensitivity, and perhaps a slower rate of aging-related decline.

In the fasted liver, SIRT1 governed two pathways to switch the way gluconeogenesis is regulated (Liu et al. 2008). Early after fasting, gluconeogenesis is activated by CREB and its coactivator CRTC2. The SIRT1 gene itself is also turned on by CREB/CRTC2 during early fasting via a site in its promoter (Noriega et al. 2011). The increase in SIRT1 then triggers deacetylation, ubiquitination, and destabilization of CRTC2, resulting in a lowering of CREB activity. At the same time, SIRT1 deacetylates and activates PGC-1 α and FOXO to facilitate an alternate pathway of activation of genes for gluconeogenesis during more prolonged fasting. It is likely that a lower magnitude of activation occurs during these later times, when ketone bodies also help bridge the energy deficit. This second mechanism may also play an important role during CR.

SIRT1 transgenic mice can show CR-like phenotypes, as discussed above. These mice were also protected against an inflammation-induced model of liver cancer (Herranz et al. 2010). The anti-inflammatory effect of sirtuins may constitute a very important function, because both SIRT1 and SIRT6 repress NF- κ B activity, as discussed above. Additional murine studies indicated that SIRT1 can be protective against colon cancer (Firestein et al. 2008) and breast cancer (Wang et al. 2008). Other studies suggest that this SIRT1 may also have oncogenic

properties in certain contexts, so the relationship between SIRT1 and cancer may not be fully understood.

SIRT1 and Neurodegenerative Diseases

Neurodegenerative diseases have become a sobering obstacle to healthy aging in humans. It is estimated that Alzheimer's disease (AD) alone will affect up to one-third of those past age 80. Methods to detect early stages of AD have become ever more sensitive, but treatments remain very elusive. Can sirtuins protect the brain? Neuronal stress, for example, in cultured neurons (Qin et al. 2006) or in the cyclin-dependent kinase-5 (cdk5) (p25) transgenic mice (Kim et al. 2007), could be mitigated by overexpression of SIRT1.

More relevant, in mice that overexpress two human genes that predispose to early AD, SIRT1 overexpression in the brain reduced the load of A β -amyloid peptide, the toxic agent in AD (Donmez et al. 2010). A β is generated by sequential proteolytic cleavages of amyloid precursor protein (APP) by β - and γ -secretases. SIRT1 had the surprising ability to activate the gene encoding the α -secretase, which cleaves APP at a different location and directs the processing of APP away from the β - and γ -secretases to reduce A β production (Donmez et al. 2010). SIRT1 activated the α -secretase gene *ADAM10* by deacetylating one of the transcription factors that binds to its promoter, the retinoic acid receptor β . The α -secretase also cleaves the membrane protein Notch, involved in neurogenesis and neuroprotection, and this may be why SIRT1 evolved to regulate *ADAM10*.

SIRT1 transgenic AD mice were protected from memory loss and plaques owing to protein aggregates of A β in the brain. AD-affected patients also show A β plaques as well as a second protein aggregate: tangles of the Tau protein in neurons. Thus, A β and Tau have been two leading candidates for the causal agents of AD. Remarkably, in a separate study in mice, SIRT1 was also demonstrated to deacetylate Tau protein to destabilize it and thereby reduce tangles (Min et al. 2010).

MITOCHONDRIAL SIRTUINS

Of the seven mammalian sirtuins, three are targeted to the mitochondrial matrix (Verdin et al. 2010), where they deacetylate or ADP-ribosylate enzymes to trigger metabolic adaptation to fuel sources such as amino acids and fatty acids during energy limitation.

SIRT3 and Metabolism

The major mitochondrial deacetylase SIRT3 promotes catabolism of acetate and fatty acids, as discussed above. Likewise, deacetylation of glutamate dehydrogenase (GDH) allows amino acids to be converted into α KG for metabolism. A recent proteomics screen identified many potential SIRT3 mitochondrial protein substrates, including ornithine transcarbamoylase (Hallows et al. 2011). Deacetylation of this urea cycle enzyme by SIRT3 activates the urea cycle for disposal of ammonia

when amino acids are catabolically stripped of their carbon. The fact that SIRT5 was already known to deacetylate and activate another urea cycle enzyme, CPS1 (Nakagawa et al. 2009; Ogura et al. 2010), reinforces the importance of the urea cycle in sirtuin-mediated adaptations to energy limitation. SIRT3 was also shown to deacetylate and activate 3-hydroxy-3-methylglutaryl CoA synthase 2 (Hmgcs2), the rate-limiting enzyme for the synthesis of the ketone β -hydroxybutyrate in the liver (Shimazu et al. 2010).

These metabolic adaptations all allow the organism to use fuels that might otherwise be stored (fat and amino acids) or ignored (acetate) and globally shift energy production away from carbohydrate catabolism. It was suggested that garnering energy from fat might per se reduce ROS production (Guarente 2008). This follows, because some of the electrons derived from oxidation of fatty acids feed into the electron transport chain via FADH₂ (and not NADH), thus bypassing one of the sources of ROS production, complex I. Of course, this strategy does not bypass the other source of ROS production, complex III. However, the induction of SIRT3 might do so (see below).

Mechanisms of Protection against Oxidative Damage by SIRT3

Beyond these metabolic functions, SIRT3 has been shown to control the levels of ROS themselves by multiple mechanisms. The implications of these findings, if confirmed in a broad range of cell types, may redirect the focus of attention back to mitochondrial oxidative damage as a primary cause of aging, unite some of the central ideas on aging, and suggest novel ways to intervene pharmacologically.

Recent studies have revealed several antioxidant functions for SIRT3 in mitochondria. The study of Someya et al. (2010) discussed above proposed a specific mechanism by which SIRT3 protected cochlea cells of the inner ear against oxidative damage during CR. SIRT3 deacetylated and activated the TCA cycle enzyme isocitrate dehydrogenase 2 (IDH2), which produces NADPH in the mitochondria. The elevated NADPH, in turn, is necessary for glutathione reductase, which converts reduced oxidized glutathione disulfide (GSSG) into glutathione, the cofactor used by mitochondrial glutathione peroxidase (GPX) to detoxify ROS. Interestingly, another target of SIRT3, GDH (Lombard et al. 2007; Schlicker et al. 2008), also produces NADPH and may contribute to the increased pool of glutathione that is available for GPX to detoxify ROS within mitochondria.

Two other studies show that SIRT3 deacetylates the critical antioxidant enzyme manganese superoxide dismutase (SOD2) in the mitochondrial matrix (Qiu et al. 2010; Tao et al. 2010). Deacetylation of SOD2 resulted in increased specific activity and enhanced scavenging of ROS. Strikingly, the two studies revealed different lysines in SOD2 that were deacetylated by SIRT3 (K53 and K68 in the Qiu et al. study and K122 in the Tao et al. study). In both studies, mutating the lysine(s) to

arginine gave rise to a hyperactive SOD2 that was not further enhanced by SIRT3, suggesting that all of these lysines may be important. The Qiu et al. study also showed that CR itself resulted in the deacetylation and activation of SOD2, presumably by up-regulating SirT3. This finding may help explain why CR has consistently been associated with a reduction in ROS levels in mitochondria (Weindruch and Walford 1988). It is important to note that the role of SIRT3 on ROS detoxification through the generation of NADPH and activation of SOD2 is confined to the mitochondrial matrix and does not represent a boost in antioxidant capacity in the nucleus or cytosol. However, SIRT3 *does* suppress ROS-responsive pathways that are induced by cardiac hypertrophy in the cytosol, such as RAS, AKT, and MAPK signaling (Sundaresan et al. 2009).

SIRT3 may do so by deacetylating numerous components of the electron transport chain itself. For example, SIRT3 was shown to deacetylate subunits of complex I (NADH dehydrogenase; Ahn et al. 2008) and complex II (succinate dehydrogenase; Cimen et al. 2009), whereas mitochondria from SIRT3^{-/-} MEFs had reduced complex I, II, and III activity (Ahn et al. 2008; Cimen et al. 2009; Kim et al. 2010). Importantly, SIRT3 suppressed ROS from complex III (Bell et al. 2011), which directs ROS to both the matrix and cytoplasm (Muller et al. 2004). Whereas SIRT3 has not been demonstrated to deacetylate subunits of the ATP generating complex V, it was shown to physically interact with complex V subunits (Law et al. 2009). The reduced steady-state ATP levels in SIRT3^{-/-} cells may result from a defect in deacetylating electron transport chain components (Ahn et al. 2008); i.e., SIRT3 deficiency may result in reduced efficiency of electron transfer within the cytochrome chain, thereby increasing the probability of electrons being transferred to molecular oxygen to generate ROS at the expense of ATP production. The fact that SIRT3 has apparently evolved multiple mechanisms to reduce ROS indicates a close relationship among this sirtuin, CR, and aging.

Both SIRT3 and SIRT4 were shown to be necessary for nutrient-sensitive protection from genotoxic stress facilitated by increased mitochondrial NAD⁺ levels (Yang et al. 2007). In addition, SIRT3 deacetylates cyclophilin D, shown to inhibit apoptosis induced by the opening of the mitochondrial permeability transition pore (Hafner et al. 2010). Reduction of the ROS burden coupled with an increase in stress resistance may maintain cells, such as the cochlea cells of the inner ear, during CR.

SIRT3, HIF-1 α , and Cancer

SIRT3^{-/-} mice were found to develop mammary tumors at 24 months of age (Kim et al. 2010). Consistent with the idea that SirT3 is a tumor suppressor, SIRT3^{-/-} mouse embryo fibroblasts (MEFs) were easier to transform (requiring a single oncogene) compared with wild-type MEFs (which also require inactivation of a tumor suppressor gene). SIRT3^{-/-} cells also showed increased superoxide levels and greater chromosome instability

when stressed. All of these findings suggested that SIRT3 suppresses tumor initiation *in vivo* by preventing chromosomal instability.

Additional insight into the role of SIRT3 as a tumor suppressor came from two studies examining the relationship between SIRT3 and the transcription factor hypoxia-inducible factor, HIF-1 α . ROS-mediated stabilization of HIF-1 α is the major adaptive response to hypoxia. In one study, the inactivation of SIRT3 by short hairpin RNA (shRNA) in several tumor cell lines gave rise to higher ROS and HIF-1 α activation in normoxia and hyperactivation of HIF-1 α in hypoxic conditions. This is consistent with the idea that SIRT3 suppresses ROS, which are necessary for the hypoxic induction of HIF-1 α . Moreover, transgenic overexpression of SIRT3 prevented the induction of HIF-1 α by hypoxia (Bell et al. 2011). This finding is interesting because HIF-1 α is known to enhance the growth of tumors by adapting them to hypoxic conditions that occur during tumor development (Majmudar et al. 2010). These adaptations include induction of aerobic glycolysis for biosynthetic intermediates (the Warburg effect; Vander Heiden et al. 2009) and induction of angiogenesis to bring additional glucose and oxygen to the tumor. In fact, xenografts of tumor cells with normal, reduced, or elevated SIRT3 levels were generated, and the tumors with reduced SirT3 were the largest, whereas the tumors with the overexpressed SIRT3 were the smallest (Bell et al. 2011). Interestingly, antioxidant treatment was able to normalize the size of the tumors lacking SirT3. Moreover, inducing SIRT3 expression *after* initiation of tumor formation sufficed to arrest tumor growth, suggesting that SIRT3 is important not only in the initiation of tumors but also in the maintenance and progression of tumors.

SIRT3^{-/-} MEFs also were shown to consume more glucose, generate more lactate, and have higher levels of glycolytic intermediates and lower levels of some TCA cycle intermediates (Finley et al. 2011). These data are also consistent with altered cellular metabolism that is similar to the Warburg effect. Consistent with this metabolic reprogramming, transcriptional profiling of SIRT3^{-/-} MEFs demonstrated hyperactivation of HIF-1 α targets, which was reversed in the presence of shRNA's targeting of HIF-1 α . Strikingly, overexpression of SIRT3 reversed the Warburg effect in different breast cancer cell lines. The above data demonstrate that SIRT3 may suppress the Warburg effect through the ROS–HIF-1 α axis. In addition, the deacetylation of cyclophilin D by SIRT3 may contribute to this suppression because it was also shown to be essential for the dissociation of hexokinase II from mitochondria (Shulga et al. 2010), thereby decreasing the entry of glucose into glycolysis. It will be important to determine how generally SIRT3-mediated control of ROS governs the Warburg effect in other kinds of tumors. Interestingly, SIRT1 was also shown to repress HIF-1 α by deacetylating it (Lim et al. 2010), illustrating a second target (along with NF- κ B) in which two sirtuins work in concert.

It is amazing at this late date that data are first emerging linking SIRT3 gene deletion and reduction in protein

levels to human breast and ovarian carcinomas (40%) and other tumors (20%–30%; Kim et al. 2010; Finley et al. 2011). These findings suggest that SIRT3 is an important tumor suppressor in human cancers. Whereas it has been suggested that aging may be the price for zealous tumor suppression early in life (e.g., by p53; Tyner et al. 2002), the above findings suggest that the tumor suppressor SIRT3 involves no such tradeoff and actually links tumor suppression to a slowing of aging.

Do SIRT1 and SIRT3 Cooperate in Calorie Restriction?

SIRT1 and SIRT3 are required for the responses to CR and may be linked by one linear pathway. This follows because the coactivator PGC-1 α and the estrogen-related receptor α were shown to bind to the SIRT3 promoter to activate transcription (Kong et al. 2010). Because SIRT1 has been convincingly shown to deacetylate PGC-1 α to increase its potential to activate transcription (Nemoto et al. 2005; Rodgers et al. 2005), it is tempting to draw a linear pathway in which CR triggers an increase in SIRT1 activity, thereby activating SIRT3 expression via deacetylation of PGC-1 α . This strategy would coordinate the CR response between the nucleus and the mitochondria and might be a core mechanism driving the anti-aging effects of this dietary regimen.

CONCLUSION

Sirtuins are regulators of metabolism in response to diet. A growing body of evidence indicates that sirtuins thus slow aging and diseases of aging, although lifespan extension has not yet been observed. This latter finding may be because SIRT1 has so many important functions in mammalian physiology that global up-regulation can exert opposing effects. Alternatively, global up-regulation may indeed slow aging globally but not affect the proximal causes, for example, lymphoma, limiting life span in the mouse strains tested.

Knocking out the mitochondrial SIRT3 (Onyango et al. 2002; Schwer et al. 2002) causes no obvious phenotypes, at least in young mice (Lombard et al. 2007). However, more subtle analyses have shown that CR induces SIRT3 levels, resulting in lower ROS and oxidative damage to mitochondria. So far, most of the evidence linking SIRT3 to these processes involves loss of function, which triggers greater ROS and mitochondrial oxidative damage and loss of protection by CR. Cell-based studies have recently provided the first evidence that gain of function has the potential to exert opposite effects (Bell et al. 2011). It will be important to expand these studies by generating transgenic mice with increased SIRT3 expression.

SIRT3 is the only sirtuin for which a human polymorphism has been reported to associate with extreme longevity. This allele creates an enhancer in intron 5 of the SIRT3 gene and is highly enriched in a long-lived population in southern Italy (Rose et al. 2003; Bellizzi et al. 2005, 2009). It will be important to carry out

comprehensive analyses of SIRT3 polymorphisms in other populations to confirm the link between SIRT3 and human longevity. More generally, genetic and physiological studies are now warranted to examine the relationship among SIRT1, and indeed all of the sirtuins, human aging, and disease.

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