Sestrins orchestrate cellular metabolism to attenuate aging

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Summary

The Sestrins constitute a family of evolutionarily-conserved stress-inducible proteins that suppress oxidative stress and regulate adenosine monophosphate-dependent protein kinase (AMPK)-mammalian target of rapamycin (mTOR) signaling. By virtue of these activities, the Sestrins serve as important regulators of metabolic homeostasis. Accordingly, inactivation of Sestrin genes in invertebrates resulted in diverse metabolic pathologies, including oxidative damage, fat accumulation, mitochondrial dysfunction and muscle degeneration that resemble accelerated tissue aging. Likewise, Sestrin deficiencies in mice led to accelerated diabetic progression upon obesity. Further investigation of Sestrin function and regulation should provide new insights into age-associated metabolic diseases, such as diabetes, myopathies and cancer.

Introduction

Sestrins are highly conserved proteins encoded by genes whose expression is upregulated in cells exposed to a variety of environmental stresses including DNA damage, oxidative stress and hypoxia. Sestrins are universally found throughout the animal kingdom, but no Sestrin homologs were identified in plants or fungi. Most vertebrates including mammals express three Sestrins (*Sesn1-3*), while most invertebrate genomes contain only a single Sestrin (*Sesn*) gene. Mammalian Sestrins are encoded by three independent genomic loci, *Sesn1-3*, and the *Sesn1* and *Sesn3* genes are subject to alternative splicing, thus generating several Sestrin proteins (Peeters et al., 2003). It has been challenging to identify biochemical functions associated with the Sestrins, partially because the proteins do not contain any known structural domains or catalytic motifs. Only a very distant sequence homology to bacterial oxidoreductases was detected, which led to discovery of Sestrins' antioxidant function (Budanov et al., 2004). Sestrins also bind to Keap1 and p62/SQSTM, and thereby suppress autophagic degradation of Keap1 and lead to enhanced Nrf2-dependent antioxidant gene transcription (Bae et al., 2013). Independently of these redox-regulating activities, Sestrins can suppress mTOR complex 1 (mTORC1) activity through the activation of

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AMPK (Budanov and Karin, 2008; Chen et al., 2010; Lee et al., 2010). Sestrins are able to bind AMPK, and through direct physical association, as well as through indirect transcriptional regulation, stimulate formation of the AMPK holoenzyme and its phosphorylation and activation by upstream kinases such as LKB1 (Budanov and Karin, 2008; Chen et al., 2010). However, the exact biochemical mechanisms through which the Sestrins function as antioxidants or as AMPK activators are unclear; protein structure determination through X-ray crystallography or nuclear magnetic resonance (NMR) would provide better clues on the physicochemical basis of Sestrins function. Although a lot of work is still needed to reveal the detailed molecular functions of the Sestrins, genetic studies have clearly shown that the Sestrins maintain metabolic homeostasis and protect cells and organisms from age-related physiological abnormalities, mainly through regulation of the AMPK-TORC1 axis. Although most of the data describing the anti-aging functions of Sestrins were initially obtained from *dSesn*-deficient *Drosophila* (Lee et al., 2010), recent results from knockout (KO) mouse strains deficient in *Sesn2* and *Sesn3* support the critical role of Sestrins in regulation of metabolism and suppression of age- and obesity-associated metabolic disorders (Bae et al., 2013; Lee et al., 2012a). Furthermore a recent study using *C. elegans* shows that *cSesn* is an important regulator of healthspan and lifespan in worms (Yang et al., 2013), suggesting that Sestrins' anti-aging function is evolutionarily conserved throughout the animal kingdom. In this review, we will discuss the physiological functions of the Sestrins with an emphasis on the regulation of metabolism and aging.

Regulation of Sestrin Expression

In response to diverse insults, cells adjust their metabolic timbre to support cellular adaptation to stress, for example by facilitating damage repair, ceasing anabolic processes and stimulating catabolic reactions. Through these adjustments, cells can prevent accumulation of damaged macromolecules and save scarce resources for diverse repair processes. Because Sestrins expression is stress-inducible (Budanov et al., 2002; Velasco-Miguel et al., 1999) the Sestrins can be involved in cellular or organism-level adaptation to diverse metabolic challenges. It is therefore critical to understand how Sestrin expression is regulated under diverse physiological and pathological contexts.

Genotoxic stress

Dysregulation of different metabolic pathways can lead to release of genotoxic compounds that damage DNA, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive carbonyl species, lipid peroxidation products and DNA-alkylating agents. Excessive genotoxic damage during metabolic stress can activate DNA damage-sensing signaling pathways, including up-regulation of tumor suppressor p53, which exerts cell cycle-inhibitory and pro-apoptotic activities as well as affecting metabolic regulation. *Sesn1* (also known as PA26), the founding member of the Sestrin family, was originally discovered as a p53-inducible gene (Velasco-Miguel et al., 1999). Different challenges, including gamma-irradiation, UV and genotoxic metabolites, stimulate transcription of the *Sesn1* and *Sesn2* genes through p53 (Budanov et al., 2002; Velasco-Miguel et al., 1999). Activation of p53 by Nutlin-3, a drug that disrupts the inhibitory association between Mdm2 and p53, can lead to induction of *Sesn1* and *Sesn2* without DNA damage (Budanov and Karin, 2008). p53 is also involved in *Sesn2* regulation by the orphan nuclear receptor TR3/Nur77 (Lee et al., 2012b). Correspondingly, p53-responsive *cis*-elements were identified in the first and second introns of the *Sesn1* gene (Velasco-Miguel et al., 1999; Wei et al., 2006) and in the first exon and 9.6kb downstream of the *Sesn2* gene (Lee et al., 2012b; Wei et al., 2006). In addition to the genotoxic challenges, paclitaxel-induced mitotic block can also induce *Sesn1* and *Sesn2* through an unknown mechanism (Rocha et al., 2011).

Hypoxia, energy deficiency and desiccation

Hypoxia, insufficient oxygen availability, is one of the most severe metabolic insults. *Sesn2* (also known as Hi95) was originally isolated as a gene activated by hypoxia in human neuroblastoma cells (Budanov et al., 2002). In many human cancer cell lines, hypoxia mimetics upregulate the expression of both *Sesn1* and *Sesn2*. Although *Sesn1* is activated strictly in a p53-dependent manner, transcriptional activation of *Sesn2* upon hypoxia is p53-independent, (Budanov et al., 2002), but is HIF-1-dependent in mouse epithelial tracheal cells (Olson et al., 2011). However, in many other cell types, *Sesn2* is induced upon hypoxia independently of HIF-1 and shows expression kinetics that are distinct from other HIF-1 target genes (Budanov et al., 2002). Presumably, in most cases, *Sesn2* transcription is induced not by hypoxia itself but as a consequence of energy deprivation caused by prolonged hypoxia. Many compounds that decrease cellular ATP concentration, such as 2-deoxyglucose (inhibitor of glycolysis) and metformin (inhibitor of mitochondrial respiration), induce *Sesn2* expression via a mechanism that is yet-to-be established (Ben-Sahra et al., 2013a). Interestingly, *Belgica antarctica*, the only insect residing in Antarctica, strongly upregulates *Sesn* expression in response to desiccation, which is considered to be critical for survival of this species in the freezing Antarctic climate (Teets et al., 2012).

Oxidative stress

Oxidative stress reflects an imbalance in ROS and RNS metabolism and impairment of the cell's ability to detoxify ROS, RNS and other reactive metabolic intermediates. All members of Sestrin family are induced by oxidative stress, although they are subject to different induction mechanisms (Figure 1A) (Budanov et al., 2004; Hagenbuchner et al., 2012; Nogueira et al., 2008). *Sesn1* is induced by hydrogen peroxide in a p53-dependent manner, whereas induction of *Sesn2* by oxidative stress is only partially p53-dependent (Sablina et al., 2005). In neurons, *Sesn2* is induced upon NMDA receptor activation, which stimulates the production of ROS, in a c/EBPβ-dependent manner (Papadia et al., 2008). More recently, oxidative stress was found to induce *Sesn2* via activation of transcription factor Nrf2 (Shin et al., 2012) and via the JNK-AP-1 signaling axis (Zhang et al., 2013). Binding sites for c/EBPβ, Nrf2 and AP-1 are all present in the *Sesn2* promoter region. *Sesn3* is stimulated by oxidative damage via activation of FoxO transcription factors (Chen et al., 2010; Hagenbuchner et al., 2012). Similarly, *dSesn* is regulated by a JNK-dFoxO signaling axis in response to chronic dTORC1-induced oxidative stress in *Drosophila* (Lee et al., 2010).

Hypernutrition, obesity and chronic mTORC1 activation

Hypernutrition and lack of exercise promote development of obesity and the metabolic syndrome, both of which have become very prevalent in modern societies. We observed that *Sesn2* is uniquely induced upon obesity in multiple mouse tissues including liver and skeletal muscle (Lee et al., 2012a). Although the mechanism of this induction response is currently elusive, it is plausible that chronic mTORC1 activation, which is associated with hypernutrition, is involved. It has been previously shown in *Drosophila* that hyperactive dTORC1 results in *dSesn* induction through a ROS-JNK-dFoxO signaling pathway (Lee et al., 2010). In several human cancer cell lines, conditions that lead to chronic mTORC1 activation, such as prolonged treatments with insulin, IGF1 or serum, also lead to increased *Sesn2* expression (Budanov, unpublished results). However, the mechanism of mammalian *Sesn2* induction upon mTORC1 activation can be different from the one responsible for *dSesn* induction. For instance, unlike *dSesn*, the only mTORC1-inducible *Sesn2* is not regulated by FoxOs in mammalian cells, whereas *Sesn1* and *Sesn3*, which are not induced upon chronic mTORC1 activation or obesity (Lee et al., 2012a), are FoxO targets (Chen et al., 2010; Greer and Brunet, 2005). Therefore, alternative transcription factors and signaling

pathways are likely to be involved in obesity- and chronic mTORC1-mediated *Sesn2* induction in mammalian cells.

Sestrins and Oxidative Metabolism

As described above, all members of Sestrin family are induced by oxidative stress, which implicates their involvement in the metabolism of ROS and other reactive metabolites. Silencing any of *Sesn1-3* by shRNA causes accumulation of ROS in various cell lines (Budanov et al., 2004; Nogueira et al., 2008), leading to DNA damage and chromosomal instability (Kopnin et al., 2007; Sablina et al., 2005) or cell death (Budanov et al., 2002; Budanov et al., 2004; Hagenbuchner et al., 2012; Nogueira et al., 2008). Inactivation of *dSesn* in *Drosophila* causes ROS accumulation and oxidative cell damage in skeletal muscle (Lee et al., 2010). Importantly, a small conserved region of the Sestrins (100-175 a.a in *Sesn1*) shows distant but traceable sequence homology to *Mycobacterium tuberculosis* AhpD protein (Budanov et al., 2004). AhpD is a critical component of the bacterial antioxidant defense system that regenerates overoxidized AhpC, a bacterial peroxiredoxin (Prx), through catalytic reduction. Similar to AhpD, the Sestrins interact with and promote regeneration of overoxidized Prx in mammalian cells (Budanov et al., 2004). Although the Sestrins do not exhibit direct catalytic activity towards Prx that leads to its reduction (Woo et al., 2009), they may promote the activity of other oxidoreductases, such as sulfiredoxin (Srx), that also regenerate Prx. Indeed, one recent study showed that Sestrins can increase Srx expression through activation of Nrf2 (Figure 1B) (Bae et al., 2013). Sestrin-dependent regulation of Prx is important for antioxidant defense in neurons and macrophages (Essler et al., 2009; Papadia et al., 2008).

Independently of their Prx-regulating activity, the Sestrins contribute to redox homeostasis through regulation of the AMPK-mTORC1 signaling pathway. Sestrins prevent mTORC1 hyperactivation that stimulates ROS production through its effects on metabolism and mitochondrial function (Lee et al., 2010). Sestrin-dependent activation of AMPK and suppression of mTORC1 activity are critical for maintaining basal autophagy (Maiuri et al., 2009). Thus, Sestrins can be important for autophagic elimination of dysfunctional mitochondria that leak electrons and produce pathogenic amounts of ROS (Ishihara et al., 2013). Correspondingly, *dSesn* deficiency in *Drosophila* results in accumulation of abnormal, ROS-producing, mitochondria in skeletal muscle (Lee et al., 2010). Importantly, ROS accumulation can be suppressed by a mutant *dSesn* that is unable to regenerate Prx but is able to inhibit mTORC1, indicating this property of the Sestrins is mediated through their effect on mTORC1. Indeed, ROS accumulation in Sestrin-deficient muscle was alleviated by pharmacological mTORC1 inhibitors (Lee et al., 2010). Sestrin-dependent inhibition of mTORC1 can be also important for autophagy-mediated degradation of Keap1, an inhibitor of Nrf2-dependent antioxidant gene expression (Bae et al., 2013). A recent report also demonstrated that, through AMPK activation, Sestrin2 can inhibit NADPH oxidase 4 (NOX4) that generates pathogenic amounts of cytosolic ROS (Eid et al., 2010; Eid et al., 2013). These findings suggest that the AMPK-mTORC1-regulating activity of Sestrins may be more physiologically significant than their Prx-regulating activities in preventing excessive ROS accumulation.

The Sestrins were also shown to mediate the antioxidant activities associated with the p53 and FoxO transcription factors (Hagenbuchner et al., 2012; Nogueira et al., 2008; Sablina et al., 2005). While high levels of oxidative stress can lead to cell death through p53- and FoxO-dependent apoptotic gene transcription, low levels of oxidative stress cause moderate activation of p53 and FoxO that can induce Sestrins to reduce oxidative stress and prevent cell death. Thus, Sestrins are important genetic components of a regulatory circuit that attenuates the detrimental consequences of oxidative stress and ensures cell viability and

function. Sestrin2-mediated oxidative stress suppression can also be important for other physiological processes, as one recent study suggests that Sestrin2's antioxidant function is important for decreasing neuropathic pain; $Sesn2^{-/-}$ mice exhibited highly increased neuropathic pain behavior after peripheral nerve injury, which was suppressible by antioxidant administration (Kallenborn-Gerhardt et al., 2013). Nevertheless, the exact role of Sestrins in oxidative stress adaptation still awaits more studies to be conducted with Sestrin-deficient mouse models.

Sestrins and Nutrient Signaling

AMPK and mTORC1 are important nutrient-sensing protein kinases that have diametrically antagonistic functions in metabolic homeostasis (Hardie et al., 2012; Zoncu et al., 2011). AMPK phosphorylates diverse anabolic enzymes, such as acetyl-CoA carboxylase (ACC), glycerol phosphate acyl transferase (GPAT), 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) and glycogen synthase (GS), directly inhibiting their enzymatic activities in glycogen and lipids biosynthesis (Hardie et al., 2012). In addition to modulating these traditional target enzymes, AMPK inhibits the transcriptional activity of sterol regulatory element binding protein (SREBP) through direct phosphorylation, thereby decreasing lipogenic gene expression (Li et al., 2011). On the other hand, AMPK can phosphorylate and activate autophagy-initiating protein kinase ULK1/2, stimulating cellular autophagic catabolism (Egan et al., 2011; Kim et al., 2011). Importantly, AMPK can also inhibit mTORC1 activity through phosphorylation-dependent activation of tuberous sclerosis complex 2 (TSC2) and subsequent inhibition of the mTORC1-activating GTPase Rheb (Inoki et al., 2003). Another report shows that AMPK can directly inhibit mTORC1 through phosphorylation-dependent inhibition of its regulatory subunit Raptor (Gwinn et al., 2008). Through these activities, AMPK stimulates cellular catabolism of sugar, protein and lipids, while inhibiting anabolism.

In contrast to AMPK, mTORC1 activates anabolic processes while inhibiting catabolic processes. mTORC1 promotes protein translation by regulating its well-characterized substrates such as p70 S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein (4E-BP) (Zoncu et al., 2011). mTORC1 can also boost cellular lipid synthesis by activating SREBP, through suppression of the SREBP inhibitor Lipin-1 (Peterson et al., 2011). mTORC1 is also an important negative regulator of autophagy that inhibits the autophagy-initiating ULK1/2 complex by direct phosphorylation (Chan, 2009; Kim et al., 2011). Recent studies suggest that mTORC1 can also stimulate synthesis of pyrimidines, which can be used as building blocks for nucleic acids (Ben-Sahra et al., 2013b; Robitaille et al., 2013). Therefore, while subjected to AMPK-mediated suppression, mTORC1 stimulates anabolic processes that are antagonized by AMPK action. However, defects in the regulatory network that modulates the AMPK-mTORC1 balance in cells can lead to aberrant activation of mTORC1, culminating in age- and obesity-associated metabolic pathologies (Howell and Manning, 2011).

When induced in response to stress, Sestrins inhibit mTORC1 through activation of AMPK (Figure 2) (Budanov and Karin, 2008). Consequently, Sestrin-deficient cells and tissues exhibit lower AMPK and higher mTORC1 activities under both normal and stressed conditions (Budanov and Karin, 2008; Lee et al., 2010; Wempe et al., 2010). In mammalian cells, Sestrin2 is found within a high molecular weight protein complex containing TSC1, TSC2 and AMPK (Budanov and Karin, 2008). Although it is not yet clear how the Sestrins activate AMPK, it has been suggested that Sestrin2 can bring an upstream kinase LKB1 and regulatory AMPKβ/γ subunits to the catalytic AMPKα subunit, thereby facilitating LKB1-dependent activatory Thr172 phosphorylation of AMPKα (Sanli et al., 2012). However, Sestrin2-dependent AMPK activation was also observed in LKB1-deficient cells, such as

HeLa cells (Wang and Karin, unpublished results), suggesting that Sestrin-induced AMPK activation can also be LKB1 independent.

Through regulation of the AMPK-mTORC1 signaling axis, the Sestrins promote metabolic adaptation of cells in response to diverse stress insults. *Sesn1* and *Sesn2*, as p53-inducible genes, mediate DNA damage-induced activation of AMPK and suppression of mTORC1 (Budanov and Karin, 2008). *Sesn1* and *Sesn2* are also responsible for mitotic arrest-induced AMPK activation and mTORC1 inhibition (Rocha et al., 2011). The inhibition of mTORC1 activity leads to hypophosphorylation of S6K and 4E-BP, ultimately resulting in cessation of protein synthesis. Thus, *Sesn1* and *Sesn2* are essential for p53-mediated suppression of protein translation during genotoxic stress (Braunstein et al., 2009; Loayza-Puch et al., 2013). Induction of *Sesn2* upon genotoxic stress can also promote autophagic catabolism, probably through the regulation of AMPK-mTORC1, enabling cells to obtain extra nutrients and energy sources (Maiuri et al., 2009). Both of these effects may be critical for suppressing cell growth during genotoxic stress and for channeling the saved energy to the DNA repair machinery, thereby promoting the survival of stressed cells. Similarly, *Sesn3*, as a FoxO-inducible protein, was shown to mediate oxidative stress-induced suppression of mTORC1 and be required for the maintenance of cellular energy stores during oxidative challenge (Chen et al., 2010). It was also shown that overexpressed *Sesn1* and *Sesn2* can protect cells from oxidative stress- and hypoxia-induced cell death (Budanov et al., 2002; Budanov et al., 2004). Therefore, the Sestrins can be viewed as stress-inducible regulators of cellular metabolism that ensure cell survival under stressful conditions.

At the organismal level, Sestrins' roles in metabolic regulation are not limited to acute stresses. In *Drosophila, dSesn* acts as a physiological feedback regulator of dTORC1 that suppresses various age-associated metabolic pathologies (Lee et al., 2010). Loss of *dSesn* causes moderate downregulation of AMPK and upregulation of dTORC1 in the fat body, thus leading to increased expression of mRNAs encoding lipogenic enzymes, ultimately resulting in triglyceride accumulation. This excessive fat accumulation can be suppressed by pharmacological activation of AMPK and inhibition of dTORC1. Similarly in mice, *Sesn2*, as an obesity-inducible Sestrin, was found to be an important suppressor of liver fat accumulation (hepatosteatosis) upon dietary or genetically-induced obesity (Lee et al., 2012a). *Sesn2* also suppresses acute hepatosteatosis induced by short-term feeding with a high-carbohydrate diet after prolonged fasting (Bae et al., 2013). Hepatosteatosis in the *Sesn2*-deficient mouse liver is associated with decreased beta-oxidation of lipids as well as with reduced mitochondrial mass and diminished hepatic autophagy. *Sesn2* was also found to regulate SREBP-1 activity; however, hepatic *de novo* lipogenesis did not change very much upon loss of *Sesn2*. Importantly, hepatosteatosis in *Sesn2*-deficient mice can be corrected by pharmacological or virus-mediated restoration of AMPK activity, supporting a role for AMPK as a major mediator of Sestrin effects on metabolism and implying that Sestrin-dependent AMPK regulation is important for liver lipid homeostasis.

Although Sestrin-dependent regulation of protein synthesis through mTORC1 can be also important for the control of cell growth and proliferation, the Sestrins do not seem to act as *bona fide* cell growth regulators. Neither *dSesn* deficiency in *Drosophila* nor *Sesn2/3* deficiency in mice results in increased body size or cell/tissue growth (Bae et al., 2013; Lee et al., 2010; Lee et al., 2012a). Nevertheless, the Sestrins' role in protein metabolism and cell growth can still be detected under conditions of mTORC1 hyperactivation, when Sestrin expression is induced. For example, in *Drosophila, dSesn* suppresses hyperactive dTORC1-induced phosphorylation of dS6K and d4E-BP and subsequent tissue overgrowth (Lee et al., 2010). Moreover, *Sesn2* suppresses cell growth and proliferation in many cancer cell lines (Budanov et al., 2002; Budanov and Karin, 2008). Therefore, it is plausible that Sestrin-dependent regulation of protein synthesis and cell growth can be important only in the

context of hyperactive mTORC1, for instance during tumorigenesis, which will be further discussed below. Collectively, these results show that Sestrins are evolutionarily conserved regulators of protein and lipid metabolism acting through the AMPK-mTORC1 signaling axis.

Sestrins Prevent Insulin Resistance and Diabetes

One of the most serious metabolic pathologies associated with chronic mTORC1 activation is insulin resistance and type II diabetes (Howell and Manning, 2011; Zoncu et al., 2011). Through its substrate S6K, mTORC1 activation results in inhibitory phosphorylation of insulin receptor substrates (IRS), thereby contributing to attenuation of insulin-induced phosphoinositide 3 kinase (PI3K)/AKT signal transduction and subsequent development of insulin resistance (Figure 3) (Um et al., 2004). Recently, it was found that mTORC1 also phosphorylates and activates growth factor receptor-bound protein 10 (Grb10), which acts as an inhibitor of the insulin-activated PI3K-AKT signaling pathway (Hsu et al., 2011; Yu et al., 2011). mTORC1-activated S6K also phosphorylates and inhibits Rictor, a component of the mTORC2 complex, that is critical for AKT activation (Julien et al., 2010; Treins et al., 2010). Chronic inhibition of autophagy in liver, which can be caused by prolonged mTORC1 activation, attenuates insulin signaling and induces insulin resistance (Yang et al., 2010). Prolonged mTORC1 activation in hepatocytes can also induce chronic endoplasmic reticulum (ER) stress (Ozcan et al., 2008), which can additionally contribute to development of insulin resistance (Ozcan et al., 2004). Thus, persistent activation of mTORC1 in response to prolonged hypernutrition can give rise to insulin resistance, elevate blood glucose and eventually drive the pathogenesis of type II diabetes.

Importantly, during obesity and also under normal conditions, *Sesn2-3* in mice and *dSesn* in *Drosophila* were all shown to be critical for maintenance of blood sugar homeostasis (Lee et al., 2012a). Especially, *Sesn2*, whose expression in liver is induced upon hypernutrition, is important for inhibition of chronic mTORC1 and suppression of insulin resistance in hepatocytes. *Sesn2* is also important for the maintenance of insulin responsiveness in adipose tissue. Consequently, *Sesn2*-deficient mice exhibit impaired glucose homeostasis upon either dietary or genetic obesity, which is mostly due to defects in insulin-mediated suppression of hepatic glucose production. Although *Sesn2*-deficient mice show no apparent defects in glucose homeostasis on a normal chow diet, concomitant deletion of *Sesn2* and *Sesn3* renders mice susceptible to spontaneous development of hepatic insulin resistance associated with elevated hepatic mTORC1 activity, even without nutritional overload or obesity. Similarly, *dSesn*-null *Drosophila* exhibit an elevated hemolymph trehalose level on a normal diet (Lee et al., 2012). These results indicate that Sestrin-dependent regulation of blood sugar homeostasis is physiologically important and evolutionarily conserved.

It has been also shown in diverse cultured cell lines that Sestrins, including *Sesn1-3* and *dSesn,* can potentiate PI3K-AKT signal transduction even in the absence of insulin (Lee et al., 2012a). Sestrin-induced activation of AKT is dependent on AMPK, TSC2 and Rictor, suggesting that Sestrins upregulate AKT signaling through AMPK and mTORC2. This further suggests that Sestrins can modulate the balance of signaling activity between mTORC1 and mTORC2 through AMPK. Indeed, Sestrins potently inhibit the mTORC1-p70S6K pathway while slightly upregulating the mTORC2-AKT pathway. This very property differentiates the Sestrins from common pharmacological mTOR inhibitors, such as rapamycin, Torin and PP242, which inhibit both mTORC1 and mTORC2 when administered for a long term. Notably pharmacological mTOR inhibitors were found to be inappropriate for treatment of obesity-associated diabetes and other metabolic pathologies because they promote insulin resistance through mTORC2 inhibition (Lamming et al., 2012). Therefore, modulation of Sestrins activity may provide an alternative approach to

prevention of obesity, insulin resistance and diabetes through attenuation of mTORC1 and potentiation of mTORC2. These findings also suggest that endogenous Sestrins are physiologically important in keeping mTORC1 activity low and mTORC2-AKT activity high, which is critical for avoiding insulin resistance and other metabolic derangements.

Sestrins Attenuate Aging

Deregulated nutrient signaling, loss of protein homeostasis and accumulation of oxidative stress are hallmarks of aging (Lopez-Otin et al., 2013). Activation of AMPK, suppression of mTORC1 and stimulation of autophagic signaling were all shown to be beneficial for extending both lifespan and healthspan (Gelino and Hansen, 2012; Harrison et al., 2009; Mair et al., 2011; Miller et al., 2011; Wilkinson et al., 2012). Thus, it seems plausible that the antioxidant, AMPK-activating, mTORC1-suppressing and autophagy-inducing abilities of the Sestrins also contribute to the attenuation of aging and suppression of age-associated diseases. Indeed, *Drosophila* and mouse models of *Sesn* deficiencies demonstrate that endogenous Sestrin activity is required to prevent diverse age- and obesity-associated pathologies. Inactivation of *dSesn* in *Drosophila* leads to chronic suppression of AMPK and activation of mTORC1, resulting in fat accumulation, blood sugar elevation, and skeletal/cardiac muscle degeneration (Lee et al., 2010). As summarized in the preceding sections, defective lipid and blood sugar homeostasis, which are hallmarks of age-associated metabolic derangements, are observed in both *dSesn*-null *Drosophila* and *Sesn2/3*-knockout mice (Lee et al., 2010; Lee et al., 2012a).

Notably, the cardiac and skeletal muscle phenotypes of the *dSesn*-null flies closely resemble the degenerative features of age-associated myopathies and cardiomyopathies (Lee et al., 2010). *dSesn*-null flies show irregularity of heart beat (arrhythmia), cardiac dilation, and decreased heart rate, ultimately leading to decreased cardiac function. The thoracic skeletal muscles of *dSesn*-null flies exhibit disorganized sarcomeric structure, dysfunctional mitochondria, accumulation of protein aggregates and oxidative stress. Interestingly, similar deterioration of cardiac/skeletal muscle is observed in flies with inactivation of ATG1, a critical regulator of autophagy, implying that regulation of autophagy is at least one mechanism that contributes to *dSesn*'s myoprotective function (Lee et al., 2010). These phenotypes observed in young *dSesn* or *Atg1* mutant flies (~20 days old) are also similar to the ones typically seen in very old WT flies (~90 days old). Many of the detrimental consequences of *dSesn* inactivation are prevented by treatment with pharmacological AMPK activators or mTORC1 inhibitors, suggesting that *dSesn* attenuates tissue aging through modulation of the AMPK-mTORC1 axis (Lee et al., 2010). The degenerative muscle phenotypes were also exhibited by mutant *C. elegans* deficient in *cSesn*, suggesting that the role of Sestrins in muscle is evolutionarily conserved (Table 1) (Yang et al., 2013). It is yet to be determined if the mammalian Sestrins are similarly involved in cardiac and skeletal muscle homeostasis during aging and other pathological conditions.

It should be noted that the DNA damage-sensing pathway composed of ATM and p53 is important for expression of endogenous Sestrins and regulation of metabolic homeostasis during aging. The *p53^Ser15Ala* knock-in mouse, which shows dramatic downregulation of *Sesn1-3* in liver, exhibits early onset hepatic insulin resistance and hyperglycemia (Armata et al., 2010). On the other hand, p53-overexpressing mice, which have elevated *Sesn1* and *Sesn2* expression in liver, show significantly increased longevity and a delayed onset of age-associated metabolic pathologies (Matheu et al., 2007). Although both studies attribute the metabolic phenotypes to the modulation of Sestrins' redox-regulating activities, it should be examined whether Sestrin-dependent regulation of the AMPK-mTOR axis provides a better explanation to the anti-diabetic and anti-aging activities of the ATM-p53 pathway. Regardless of the molecular mechanisms operating downstream of the Sestrins, it is highly

likely that the Sestrins mediate the metabolic output of the DNA damage-sensing pathway, which is abrogated during tumorigenesis, aging and metabolic pathologies.

Notably, current data from *Drosophila* and *C. elegans* indicate that the association between Sestrin and lifespan is not as strong as the antagonistic relationship between Sestrin and age-associated pathologies. We and others observed that the lifespan of *dSesn*-null flies under normal conditions is not significantly different from that of WT flies (Lee and Karin, unpublished results; P. Kapahi, personal communication). The lifespan of *cSesn*-null worms was only slightly shorter than that of WT worms (Yang et al., 2013). This weak association between Sestrins and lifespan is in stark contrast to the robust linkage between Sestrins and age-associated metabolic pathologies. However, according to recent studies, most (if not all) of the age-associated deaths in flies and worms are caused by, or associated with, intestinal disintegration (McGee et al., 2011; Rera et al., 2012). As Sestrins may not play major roles in regulating intestinal barrier function, it is not very surprising that Sestrin-deficient invertebrates show almost normal lifespan. However, because Sestrin mutants show strong age-associated pathologies in other metabolic organs such as fat body, liver and skeletal/cardiac muscle in virtually all of the model organisms examined, including *C. elegans, Drosophila* and mice, Sestrins still can be considered as an important determinants of healthspan, if not lifespan. It should be also noted that, in mammals, the cause of age-related death is different from invertebrates: cardiac dysfunction and malignant neoplasms are the most significant causes of age-related death in humans. Cardiac dysfunction in *Drosophila* is not immediately associated with organismal death (Ocorr et al., 2007) and *C. elegans* even does not have a distinct cardiac structure. Death caused by malignancy also does not normally occur in flies or in worms. Because Sestrins are suppressors of age-associated cardiac pathologies and tumorigenesis, it is still plausible that Sestrins regulate lifespan as well as healthspan in mammals.

Sestrins and Cancer

Advanced age is the major risk factor for cancer. As a result, cancer is one of the most significant causes of age-related human death. Cancer is a disease tightly linked to metabolic dysregulation, oxidative stress and genomic instability. Given that Sestrins are important for suppressing oxidative damage, inactivation of Sestrins and accumulation of ROS and RNS might contribute to carcinogenesis. Accordingly, silencing of Sestrins in cancer cells stimulates mutagenesis, genomic instability and growth of tumor xenografts; features that are largely eliminated by antioxidant treatment (Sablina et al., 2005). In addition, as suppressors of mTORC1 activity, Sestrins can inhibit cancer cell growth and therefore may be preferentially lost during tumorigenesis. Indeed, *Sesn1* and *Sesn2* are generally downregulated in cancer, probably due to the inactivation of their master regulator-p53 (Loayza-Puch et al., 2013). Constitutively active *Ras* oncogene also downregulates *Sesn1 and Sesn3* (Kopnin et al., 2007). Although the mechanism of *Ras*-induced suppression has not been determined, it has been recently shown that the transcription factor HSF1 mediates *Sesn3* regulation by *Ras* (Zamkova et al., 2013). Loss-of-heterozygocity in *Sesn1* (6q21) and *Sesn2* (1p35) is also often observed in diverse human cancers (Ragnarsson et al., 1999; Velasco-Miguel et al., 1999). A mutation in the *Sesn2* gene (Pro87Ser) was recently identified as a cancer-driving mutation in myeloproliferative neoplasms through genome sequencing (Hou et al., 2012), although how this mutation specifically affects Sestrin2 function is not yet known. Another mechanism that can lead to Sestrin depletion has been described in endometrial cancers where *Sesn3* is heavily methylated in 20% of tumors (Zighelboim et al., 2007).

Despite their involvement in tumor suppression and genome protection, Sestrins are still expressed in many cancers (Budanov et al., 2002) and might actually be required for

maintaining the viability of cancer cells under certain conditions. Very high levels of oxidative stress, associated with chronic inflammation, prolonged growth factor signaling and dysfunctional mitochondria, can be detrimental for the survival and propagation of cancer cells. For this reason, many cancer cells upregulate the expression of antioxidant proteins, and Sestrins are part of the antioxidant defense system. Another potential benefit to keep Sestrin function intact is activation of autophagy (Ishihara et al., 2013; Maiuri et al., 2009), which can support tumor growth and metabolism during conditions of limited nutrient and oxygen supply. Finally, Sestrin-dependent activation of mTORC2-AKT (Lee et al., 2012a), a well recognized oncogenic pathway, might contribute to carcinogenesis through promotion of cancer cell proliferation and survival.

Being stress-responsive genes, Sestrins can also contribute to the effects of cytotoxic anticancer therapies. Sestrins were shown to promote DNA damage-induced cell death upon irradiation and genotoxic drug treatments through a poorly understood mechanism that may be related to the inhibition of mTORC1 (Sanli et al., 2012). On the other hand, Sestrins may hamper oxidative damage-associated chemotherapy by promoting cancer cell survival (Budanov et al., 2002; Budanov et al., 2004; Hagenbuchner et al., 2012). Furthermore, it is also possible that hypoxia-induced *Sesn2* may contribute to the well-known resistance of hypoxic cancer cells to radiotherapy and chemotherapy. Thus the Sestrins might be important modulators of the outcome of cancer therapy, although whether they are advantageous or disadvantageous would have to be validated in various cancer models. In any case, it is probable that Sestrins may serve as predictive markers for the efficiency of cancer therapy and help choose the best strategy for treatment. Modulation of Sestrin expression or activity by small molecules might improve the efficiency of radiotherapy or chemotherapy.

Unanswered Questions and Future Directions

Apparently, there are many remaining and unanswered questions regarding Sestrin biology and biochemistry. First and foremost, the chemical basis of Sestrins' antioxidant and AMPK-activating/mTORC1-inhibiting activities should be clarified. Elucidation of Sestrin structure and determination of critical amino acid residues should be one of the first steps towards a better understanding of the biochemical properties of Sestrin proteins. These studies would also facilitate future development of Sestrin-mimicking small molecules. Second, we need to understand how Sestrin expression is regulated in response to metabolic stressors, such as hypernutrition, energy depletion and ER stress. Third, the detailed molecular mechanisms through which Sestrin induction normalizes metabolic derangements during obesity and normal aging should be determined. Fourth, the role of mammalian Sestrin1 needs to be genetically determined. Because Sestrin1 is highly enriched in skeletal muscle (Peeters et al., 2003), it is plausible that it may be analogous to cSestrin and dSestrin in control of muscle homeostasis (Lee et al., 2010; Yang et al., 2013). However, to test this hypothesis, muscle-specific *Sesn1*-knockout mice are needed. It would be also interesting to see if Sestrin1 and other mammalian Sestrins are required for preventing cardiac pathologies, as in *Drosophila* (Lee et al., 2010). Finally, it is important to determine and evaluate the impact of mammalian Sestrins on the regulation of aging, carcinogenesis, the metabolic syndrome and other diseases. The recently generated Sestrin-deficient mouse strains would provide important tools for determining the role of each Sestrin in diverse physiological contexts. Through these future studies, we will be able to understand how Sestrins regulate aging and metabolism in mammals and whether their antioxidant function, effects on AMPK-mTORC1 signaling, or some yet-to-be-defined activities are most important.

Conclusion

Chronic stress, metabolic derangements and organ dysfunction are hallmarks of aging, obesity and their co-morbidities. Formerly discovered as stress-responsive and p53-regulated proteins, the Sestrins are now recognized as important regulators of metabolism that ensure physiological homeostasis, suppression of age- and obesity-associated diseases, muscle degeneration, cardiac malfunction and cancer. Consequently, the Sestrins may be imminent therapeutic targets for attenuation of aging and prevention of cancer and obesity-related metabolic derangements.

Acknowledgments

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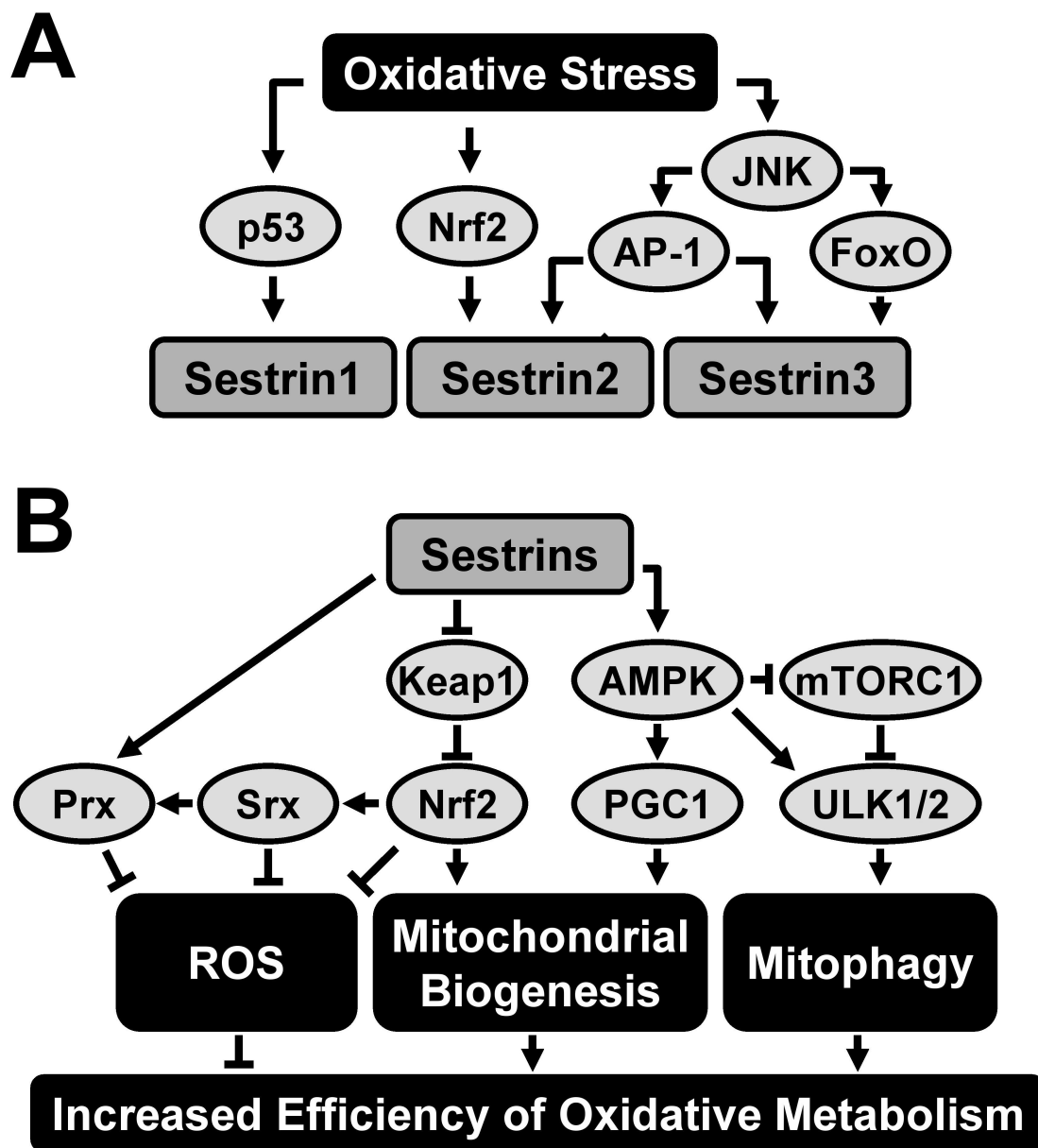
A

Oxidative Stress

p53 Nrf2 JNK AP-1 FoxO

Sestrin1 **Sestrin2** **Sestrin3**

B

Sestrins

Keap1 AMPK mTORC1

Prx ← Srx ← Nrf2 PGC1 ULK1/2

ROS **Mitochondrial Biogenesis** **Mitophagy**

Increased Efficiency of Oxidative Metabolism

Figure 1. Regulation of Oxidative Metabolism by Sestrin-Family Proteins
(A) Regulation of Sestrin expression by oxidative stress. Although p53 is essential for Sestrin1 expression after oxidative stress, it is dispensable for induction of Sestrin2 and Sestrin3. While Nrf2 and AP-1 are required for Sestrin2 induction, FoxO1 and FoxO3 are required for Sestrin3 induction upon oxidative stress. (B) Signaling pathways through which Sestrins control oxidative stress. Sestrins can recycle peroxiredoxin (Prx) as a part of an oxidoreductase enzyme complex that includes sulfiredoxin (Srx). Alternatively, Sestrins activate an antioxidant transcriptional program by stabilizing Nrf2 through removal of its inhibitor Keap1. Sestrin-induced AMPK activation can lead to activation of PPARγ coactivator 1α (PGC1α) resulting in increased mitochondrial biogenesis, whereas Sestrin-mediated AMPK activation can lead to upregulation of autophagy that removes

dysfunctional mitochondria (mitophagy). Through these activities Sestrins decrease ROS accumulation and stimulate anti-oxidant defenses.
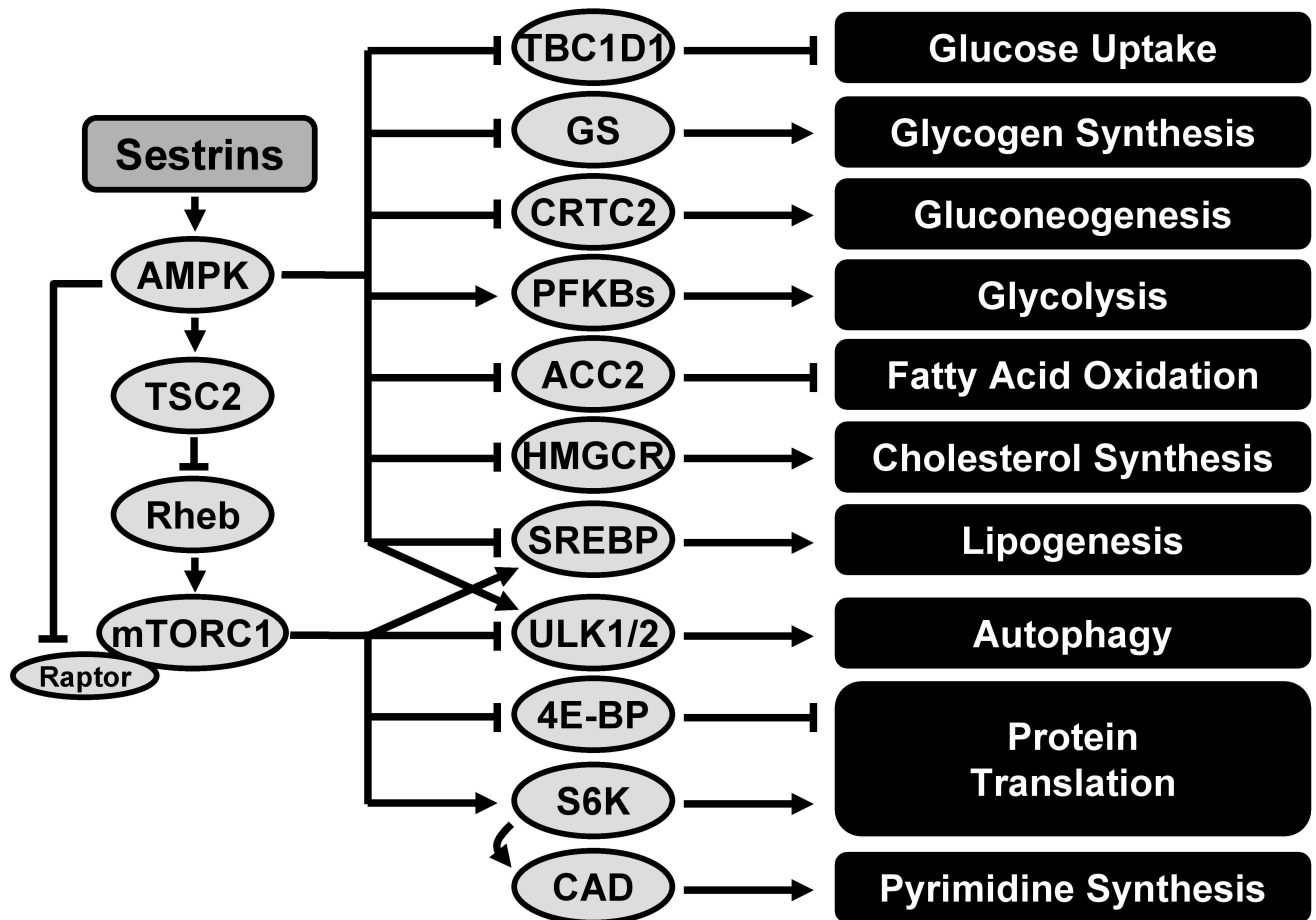
Figure 2. Regulation of Nutrient-Sensing Signaling Pathways by Sestrins
Sestrins control metabolism through AMPK and mTORC1. Sestrins potentiate AMPK activation and thereby suppress mTORC1 activity, leading to inhibition of cellular anabolism and augmentation of catabolic processes such as beta-oxidation and autophagy. Abbreviations: TBC1D1, TBC1 domain family member 1; GS, glycogen synthase; CRTC2, CREB regulated transcription coactivator 2; PKFB, fructose-6-phosphate kinase; ACC2, acetyl-coA carboxylase 2; HMGCR, HMG-CoA reductase; CAD, carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase.
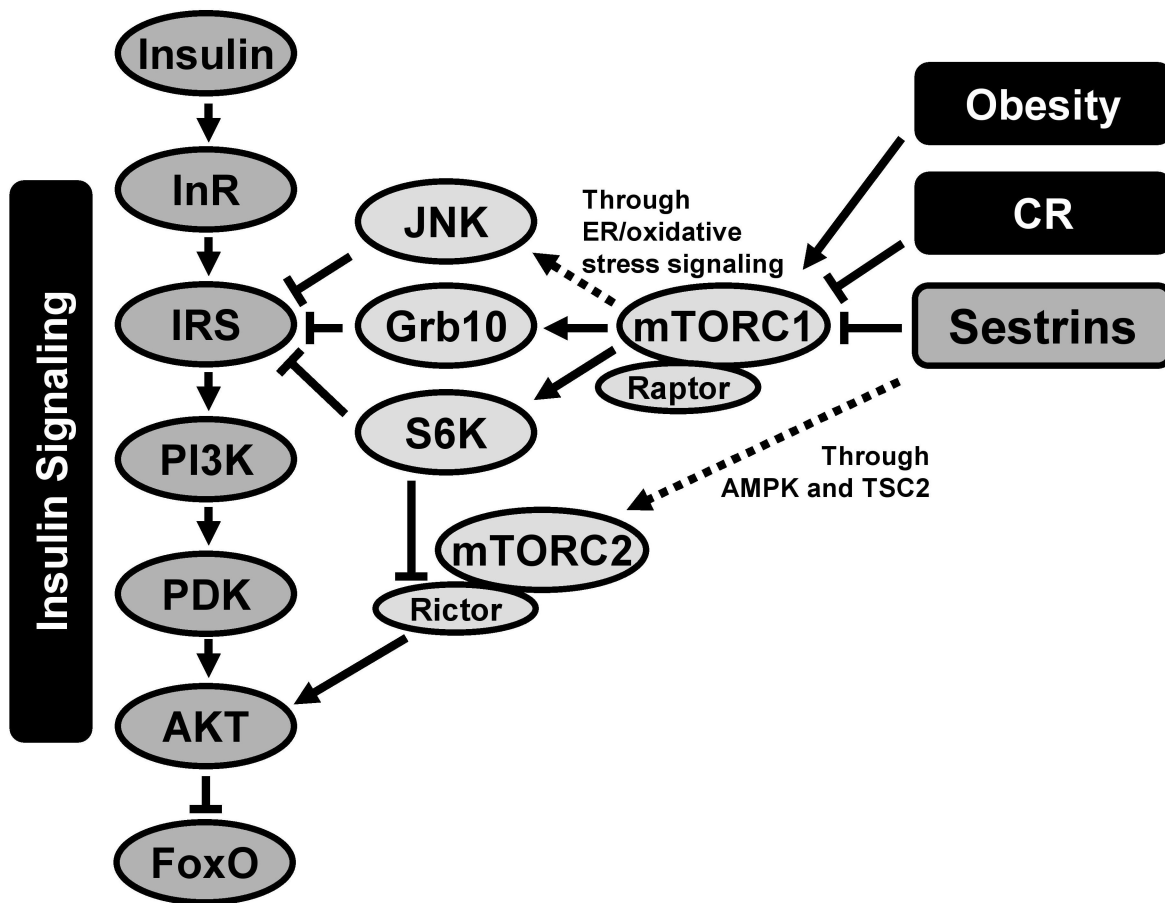
Figure 3. Regulation of Insulin Signaling by Sestrins
Sestrin-enhanced activation of AMPK liberates insulin signaling from mTORC1-mediated inhibitory effects. Abbreviations: CR, caloric restriction; InR, insulin receptor; IRS, insulin receptor substrate, PDK, phosphoinositide-dependent kinase; Grb10, Growth factor receptor-bound protein 10.

Table 1
Phenotypes of Sestrin-Modified Genetic Model Organisms

Genetic studies done in C. elegans (Yang et al., 2013), Drosophila (Edwards et al., 2009; Lee et al., 2010) and mice (Bae et al., 2013; Kallenborn-Gerhardt et al., 2013; Lee et al., 2012a; Wempe et al., 2010) suggest that Sestrin-family proteins are critical for suppressing age- and obesity-associated metabolic pathologies in diverse organ systems. Abbreviations: COPD, chronic obstructive pulmonary disease; HFD, high fat diet.

Organism	Gene(s) mutated	Nature of mutation(s)	Context	Phenotype	Reference
C. elegans	cSesn (Sesn-1 or Y74C9A.5)	Loss of function	Normal aging	Reduced lifespan	Yang et al., 2013
				Reduced locomotor activity	
				Increased muscle ROS	
				Muscle actin disorganization	
			Bacterial infection	Normal resistance	
			Oxidative stress (H_2O_2)	Reduced resistance	
			Heat stress (35°C)	Reduced resistance	
			Heavy metal stress ($CuSO_4$)	Reduced resistance	
		Gain of function	Normal aging	Increased lifespan	Yang et al., 2013
				Increased locomotor activity	
				Decreased muscle ROS	
			Oxidative stress (H_2O_2)	Increased resistance	
			Heat stress (35°C)	Increased resistance	
			Heavy metal stress ($CuSO_4$)	Increased resistance	
Drosophila	dSesn (Sesn or CG11299)	Loss of function	Normal aging	Less aggressivity	Edwards et al., 2009
				Increased fat accumulation	Lee et al., 2010
				Cardiac dilation	
				Decreased/arrhythmic heartbeat	
				Muscle degeneration	
				Increased muscle ROS	
				Protein aggregate formation	
				Increased mTORC1 signaling	
		Gain of function	Developing imaginal disc	Growth suppression	Lee et al., 2010
				Decreased mTORC1 signaling	
Mouse	Sesn2 (Hi-95)	Loss of function	Normal aging	Normal body weight	Lee et al., 2012
				Normal glucose tolerance	
				Normal insulin sensitivity	
				Normal liver fat accumulation	
				Normal mTORC1 signaling	
			Nerve injury	Increased ROS accumulation	Kallenborn-Gerhardt et al., 2013
				Increased neuropathic pain	
			Mouse model of COPD	Reduced emphysema	Wempe et al., 2010
				Increased ROS accumulation	

Organism	Gene(s) mutated	Nature of mutation(s)	Context	Phenotype	Reference
				Increased mTORC1 signaling	
				Reduced TGFβ signaling	
			Fasting	Reduced autophagy	Bae et al., 2013
			Fasting/Refeeding	Increased hepatosteatosis	
				Increased ROS accumulation	
				Increased liver damage	
				Increased mTORC1 signaling	
			HFD-induced obesity	Body weight same as WT (con)	Lee et al., 2012
				Increased hepatosteatosis	
				Increased glucose intolerance	
				Increased insulin resistance	
				Increased mTORC1 signaling	
			Lep^{ob}-induced obesity	Body weight same as WT (con)	
				Increased hepatosteatosis	
				Increased glucose intolerance	
				Increased insulin resistance	
				Increased mTORC1 signaling	
	Sesn2 & Sesn3	Loss of function	Normal aging	Unaltered body weight	Lee et al., 2012
				Increased glucose intolerance	
				Increased insulin resistance	
				Increased mTORC1 signaling	