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AMPK at the Nexus of Energetics and Aging

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Abstract

When energy supply is low, organisms respond by slowing aging and increasing resistance to diverse age-related pathologies. Targeting the mechanisms underpinning this response may therefore treat multiple disorders through a single intervention. Here we discuss AMP-activated protein kinase (AMPK) as an integrator and mediator of several pathways and processes linking energetics to longevity. Activated by low energy, AMPK is both pro-longevity and druggable, but its role in some pathologies may not be beneficial. As such, activating AMPK may modulate multiple longevity pathways to promote healthy aging, but unlocking its full potential may require selective targeting towards substrates involved in longevity-assurance.

Introduction

Advances in public health have dramatically increased the number of people surviving into old age (Christensen et al., 2009). Although human populations worldwide are living longer, however, we are not necessarily aging gracefully. Patient age is the single biggest risk factor for the majority of complex diseases, including cancer, metabolic disease and neurodegenerative disorders. Current biomedical strategy is to treat each of these pathologies in separation, targeting their unique proximal causes to alleviate them. Unfortunately, the prevalence of comorbidity in the elderly limits the impact of such a strategy; two-thirds of the elderly in the United States have multiple chronic diseases (CDC, 2013). Therefore, even substantial progress on a single disease may have only marginal effects on overall disease-free healthspan (Goldman et al., 2013).

An alternative approach to tackle age-onset pathologies is to target their commonality: the aging process itself. Although aging has long been appreciated as a risk factor, modern medicine has largely ignored it as a therapeutic target. Aging seemed simply too complex a phenotype to study, being the result of multiple genetic and environmental factors too intricate to untangle. This paradigm was irreversibly shifted in the late 20th century by elegant experiments in the nematode *C. elegans*, which demonstrated that single gene mutations could dramatically prolong lifespan and maintain animals in a youthful state

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(Kenyon et al., 1993). Conservation of these genetic effects in fruit flies, mouse, and long-lived human populations gave birth to a new field: the genetics of aging (Fontana et al., 2010). The overriding premise is that unlike chronological age, physiological age is malleable, and mechanisms that modulate it can be exploited to reduce overall disease risk (Figure 1).

Dietary restriction (DR), the reduction of food intake without malnutrition, serves as a physiologically relevant proof-of-principle that a single intervention can provide broad-spectrum disease benefits. DR elicits a physiological 'low energy' state, and this switch, mediated by conserved nutrient-sensing pathways, delays aging in species ranging from yeast to primates (Mair and Dillin, 2008). DR confers protection to multiple age-related diseases including neurodegenerative disease, kidney disease, autoimmune disease, stroke, osteoporosis, glaucoma, cardiovascular disease, metabolic disease, and many forms of cancer (Masoro, 2002). Understanding and targeting molecular mediators of DR might allow us to harness its therapeutic potential while bypassing dietary interventions and the negative physiological consequences of low energy. In this review we will discuss evidence for and against AMP-activated protein kinase (AMPK) as a conserved link between energetics and longevity, including the tripartite effects of AMPK on aging-related pathways, processes and pathologies.

AMPK

AMPK is highly conserved across eukaryotes and serves as an ancestral energy sensor (Hardie et al., 2012). When cellular energy is low, AMPK is activated and targets a range of physiological processes, the net response of which is an increase in energy production and a coordinated decrease in ATP usage. AMPK exists as a heterotrimer comprised of a catalytic α subunit with regulatory β and γ subunits (Xiao et al., 2011). Phosphorylation of the α subunit at Thr-172 is required for the kinase activity of AMPK (Hawley et al., 1996) and activates its kinase activity greater than 100-fold. The major upstream kinase targeting this site is Liver Kinase B1 (LKB1) (Hawley et al., 2003; Shaw et al., 2004; Woods et al., 2003), though two other AMPK kinases have also been identified: Calcium/calmodulin-dependent kinase kinase β (CaMKKβ) (Hawley et al., 2005; Woods et al., 2005) and transforming growth factor-β-activated kinase 1 (Tak1) (Momcilovic et al., 2006). In the context of energy stress, LKB1 is the predominant AMPK-Kinase (Tsou etal., 2011).

Nucleotide binding also acutely tunes AMPK to cellular energy status. The γ subunit contains four potential nucleotide-binding sites, two of which can bind AMP, ADP, or ATP in a competitive manner. Under low energy conditions the AMP/ADP:ATP ratio increases and AMP/ADP binds to AMPK in place of ATP (Xiao et al., 2011). This nucleotide binding exchange has a three-pronged effect on AMPK activity. Binding of AMP allosterically activates AMPK 2-10 fold and promotes α Thr-172 phosphorylation by LKB1 (Gowans et al., 2013), while both AMP and ADP protect AMPK against dephosphorylation at α Thr-172 (Oakhill et al., 2011; Xiao et al., 2011), most likely by inducing conformational changes that inhibit phosphatase access.

Given the conservation of both AMPK and the DR response, AMPK makes an appealing mechanistic link between energy status, DR and lifespan. Supporting this idea, lifespan extension in the nematode *C. elegans* via dilution of the *E. coll* food source on solid growth media requires one of the two *C. elegans* catalytic subunits of AMPK, AAK-2 (Greer et al., 2007a). Reducing glycolysis and glucose metabolism by feeding *C. elegans* 2-deoxy-D-glucose also increases lifespan in an *aak-2* dependent manner (Schulz et al., 2007). Furthermore, *aak-2* is required for the extended lifespan seen when *C. elegans* deprived of food enter a diapause-like arrest in either an early larval stage (Fukuyama et al., 2012) or an alternate 'dauer' development stage (Narbonne and Roy, 2009). In a *Drosophila* study employing a DR protocol where only the yeast component of the fly diet is reduced, restoring AMP:ATP ratio by supplementing the animals with adenine completely blocks lifespan extension, while the delayed aging seen in AMP biosynthesis mutants is suppressed by dominant negative AMPK (Stenesen et al., 2013). Importantly, the term 'DR' is used to describe a variety of regimens, including changes in caloric intake, nutritional composition or food availability. Though multiple DR paradigms activate AMPK in worms (Brunet, personal communication), AMPK is not required for lifespan extension by all of them. For example, *aak-2* is not required for lifespan extension in worms fed diluted bacteria in liquid culture, despite robust lifespan extension (Greer and Brunet, 2009; Mair et al., 2009). Why AMPK is not universally required deserves further exploration. Explanations could involve employment of different AMPK isoforms or secondary signaling events elicited under the alternative metabolic or environmental conditions.

The majority of mammalian data describing effects of food intake on AMPK activity comes from work on acute starvation, and whether chronic DR activates AMPK is dependent upon the DR protocol used and the tissue examined (Canto and Auwerx, 2011). Increases in DR severity from 5% to 40% induce progressive activation of AMPK in rat liver and mammary carcinomas (Jiang et al., 2008). Long term DR also increases AMPKα Thr172 phosphorylation in mouse heart (Edwards et al., 2010) and skeletal muscle (Palacios et al., 2009). However, examples exist where chronic DR fails to activate AMPK (Gonzalez et al., 2004) in mouse and even suppresses it in rat liver (To et al., 2007). As in worms, basic husbandry regimes and DR protocols vary between groups studying DR in flies, mice and monkeys alike (Mair and Dillin, 2008), and more work is needed to determine the reliance of these differing regimes on AMPK.

AMPK Is a Pro-longevity Kinase

Activating AMPK is sufficient to extend lifespan in model organisms. Increasing expression of *aak-2*, in *C. elegans* increases lifespan by 13% and mimics DR in well-fed animals (Apfeld et al., 2004). Similar increases in lifespan are also seen in transgenic worms expressing a modified AMPK-γ subunit (Greer et al., 2007a), in which the AMP binding pocket is mutated at conserved residues analogous to mutations that induce constitutively active AMPK in mammals (Arad et al., 2002). More recently, a 38% median increase in lifespan was shown in *C. elegans* expressing a truncated form of *aak-2* containing only the catalytic domain, which uncouples its activation from nucleotide binding (Mair et al., 2011). AMPK is highly conserved across eukaryotes (Hardie, 2007), and this conservation applies to its ability to modulate lifespan. Over-expression of the single *Drosophila* AMPK-α

subunit in either muscle or the fat body extends lifespan in the fruit fly (Stenesen et al., 2013), as does overexpression of LKB1 (Funakoshi et al., 2011).

Anti-Aging Small Molecules and AMPK

AMPK is activated by a surprisingly wide array of small molecules, increasing its appeal as a longevity target. However, as we outline here, many of these effects are indirect, and work in model organisms is yet to fully elucidate the tissue and cell types AMPK acts in to promote lifespan. Indeed, activation of AMPK in certain tissues or conditions might in fact be damaging to health (see AMPK and Aging Pathologies), highlighting a need to selectively activate AMPK or target specific substrates if we are to translate work in model systems to promote human healthy aging.

Metformin

Many classical agonists activate AMPK indirectly by altering the AMP/ADP:ATP balance in the cell. Such drugs include the biguanides metformin and phenformin, which disrupt mitochondrial function by inhibiting electron transport chain complex I, and 5-aminoimidazole-4-carboxamide-1-D-ribo-furanoside (AICAR), which cells convert to an AMP analog, ZMP. Although these compounds promote longevity in some settings, the direct role of AMPK remains unclear. The beneficial effect of metformin on health is more robust than its effects on aging *per se*. Metformin is one of the most widely prescribed treatments for type II diabetes and patients taking metformin show protection against cancer independently of its effects on diabetes (Gallagher and Leroith, 2011). However, despite frequent use of metformin as an AMPK agonist in biochemical studies, whether AMPK is actually the key mediator of the effects of metformin on health is uncertain. Metformin can potentially bind directly to AMPK (Zhang et al., 2012), but a more accepted paradigm is that AMPK is activated as a secondary effect of metformin perturbing mitochondrial electron transport chain function (Hardie, 2006). Metformin can also exert physiological effects independent of AMPK. Metformin can inhibit TOR Complex I (TORC1) in the absence of AMPK (Kalender et al., 2010), and block glucose production in mouse primary hepatocytes lacking both AMPK catalytic subunits (Foretz et al., 2010), via suppression of PKA and glucagon (Miller et al., 2013).

C. elegans fed 50 mM metformin maintain a youthful state and display a 50% increase in median lifespan that is dependent on *aak-2* (Onken and Driscoll, 2010). Interestingly, unlike genetic activation of AMPK (Greer et al., 2007a), the effects of metformin on longevity do not require FOXO/DAF-16, a pro-longevity transcription factor activated by reduced insulin/IGF-1 signaling (rlIS) (Kenyon, 2010). Instead, metformin-induced longevity in the worm depends upon the ortholog of mammalian Nrf2, SKN-1, which also plays a role in lifespan extension by DR (Bishop and Guarente, 2007). Common husbandry procedure for *C. elegans* is to co-culture them with live *E. coli* that act as a food source for the worm, and this ‘two organism’ situation can present problems in small molecule studies. Elegant data from (Cabreiro et al., 2013) show that metformin indeed extends *C. elegans* lifespan via AMPK, but does so indirectly by altering folate metabolism in the live *E. coli*. Metformin fails to increase lifespan of worms cultured on dead bacteria.

Whether the effect of metformin on aging in other species also functions via the microbiome is an area worthy of exploration (Heintz and Mair, 2014). Indeed, the effect of metformin on longevity itself in organisms other than *C. elegans* is less than clear. Despite robustly activating AMPK and reducing stored triacylglycerides (TAGs), metformin does not increase *Drosophila* lifespan (Slack et al., 2012). Failure to promote longevity may occur because ubiquitous activation of AMPK in the fly is detrimental, or that metformin fails to induce AMPK in the relevant tissues for longevity. Much of the work examining the effects of AMPK agonists on rodent lifespan has been performed on short-lived strains pre-disposed to cancer, and in this context both phenformin and metformin increase lifespan (Anisimov et al., 2005; Dilman and Anisimov, 1980). Effects in 'wild type' rodent strains are mixed. Metformin does not increase lifespan of Fisher-344 rats (Smith et al., 2010), but a recent report shows beneficial effects of chronic metformin feeding starting in middle-aged C57BL/6 mice (Martin-Montalvo et al., 2013). The effects on longevity in this case are dose-dependent and small: 1% dietary metformin shortens lifespan, while 0.1% increases median lifespan of male mice by 5%. The body of metformin data thus holds promise—especially regarding healthspan as opposed to lifespan—but reveals an abundance of off-target effects, providing a clear example of the need for target and tissue specificity in the search for small-molecule DR mimetics.

Resveratrol

Resveratrol is a polyphenol that improves metabolic fitness of mice and monkeys fed a high-fat diet (HFD) (Baur et al., 2006; Jimenez-Gomez et al., 2013). Resveratrol was first shown to increase replicative lifespan of budding yeast (Howitz et al., 2003), and its effect on aging is conserved. Resveratrol increases lifespan in *C. elegans*, *Drosophila* (Wood et al., 2004), and mice on a HFD (Baur et al., 2006) in a manner suggested to function via the NAD-dependent deacetylase Sir2p and its orthologs. Following initial discoveries, however, both the ability of resveratrol to directly agonize Sir2p (Kaeberlein et al., 2005) and the robustness of its effects on lifespan in lower organisms were challenged (Bass et al., 2007), leading to speculation on whether the physiological effects of resveratrol might be mediated by alternative targets. An additional consequence of resveratrol administration is activation of AMPK (Baur et al., 2006), and this mechanism may mediate some of its beneficial effects on health. Mice lacking either AMPK α 1 or 2 have severely blunted responses to resveratrol, and resveratrol activates AMPK in mouse embryonic fibroblasts lacking SirT1 function (Um et al., 2010). Furthermore, AMPK can indirectly activate the mammalian Sir2p ortholog, SirT1, by altering the NAD/NADH ratio (Canto et al., 2009), suggesting an alternative indirect route for sirtuin activation by resveratrol.

Recent data attempting to reconcile the requirement of SirT1 or AMPK activation for the effects of resveratrol report critical effects of dose. Though resveratrol activates AMPK at all concentrations, this effect is SirT1-dependent at low doses, but independent at higher doses (Price et al., 2012). As such, the relevant contributions of these two fuel sensors to any effects of resveratrol on longevity may be context-specific. Recent work by Hubbard et al. (2013) demonstrates that resveratrol can allosterically activate SirT1 in a mechanism that requires a single amino acid, Glu^{230}. Whether this allosteric interaction is required for the effects of resveratrol and other sirtuin-activating compounds on longevity requires

evaluation. Irrespective of whether the beneficial effects of resveratrol require AMPK and SirT1 to function epistatically or in concert, it is becoming increasingly clear that AMPK plays a key role. Indeed, comparison of whole genome transcriptional changes induced by resveratrol (Viswanathan et al., 2005) and genetic activation of AMPK (Mair et al., 2011) in nematodes reveals striking overlap (Rodrigues-Grant & Mair, unpublished), suggesting that their effects on healthy aging function via similar mechanisms.

Rapamycin, Aspirin, and Direct AMPK Agonists

The National Institute on Aging recently implemented an intervention-testing program (ITP) to evaluate the ability of candidate small molecules to prolong lifespan of genetically heterogeneous mice. The highlight of the ITP to date has been the lifespan extending effects of late onset supplementation of rapamycin (Harrison et al., 2009). Rapamycin, a macrolide originally used as an immunosuppressant for recipients of organ transplantation, acts primarily through inhibition of TORC1. In worms, flies and mammals, rapamycin prolongs lifespan and protects against several age-related diseases (Johnson et al., 2013; Lamming et al., 2013). Suppression of mTOR is one of the key outputs of AMPK (discussed below), and rapamycin may therefore phenocopy some effects of AMPK. The ITP is also currently testing aspirin, which has been shown to increase worm lifespan (Ayyadevara et al., 2013) dependent upon AMPK (Wan et al., 2013). Interestingly, aspirin is rapidly metabolized to salicylate, which was recently shown to directly agonize AMPK (Hawley et al., 2012). Salicylate binds to AMPK β1, likely interacting with the same site as the direct agonist A-769662 (Cool et al., 2006). It should therefore prove informative if specific activators of AMPK such as salicylate, A-769662 or the recently reported ‘C24’ (Li et al., 2013) have similar beneficial effects on lifespan as indirect agonists such as resveratrol and metformin.

AMPK and Aging Pathways

AMPK's appeal as a target to promote healthy aging stems in part from its ability to integrate multiple signaling and transcriptional pathways known to promote longevity (Figure 2). However, pervasiveness of feedback regulation within this network makes assignment of components into linear pathways both challenging and sometimes overly simplistic. This is especially the case for aging research. Because invertebrates facilitate rapid genetic analysis and have shorter lifespans, longevity mechanisms are typically drawn from linear genetic epistasis of invertebrate models. Conversely, detailed signaling mechanisms are often gleaned from mammalian tissue culture and extrapolated back to invertebrates with little validation. Here we review both genetic and biochemical links between AMPK and key longevity modulators across models.

TOR/S6K

As an intracellular nutrient-sensor, TOR integrates amino acid sensing with upstream signals from growth factor signaling pathways. The effect of TOR activation is a coordinated stimulation of cellular processes that promote growth when nutrients are abundant. AMPK and TOR therefore show opposing responses to low cellular energy/nutrient status, being activated or repressed, respectively. Interestingly, AMPK and TOR also show antagonistic effects towards many shared downstream processes, including autophagy, lipid metabolism, and protein synthesis (Hardie et al., 2012; Laplante and Sabatini, 2012).

Given that AMPK and TOR are antagonistically regulated by nutrients, one might expect that they have opposite effects on aging, and indeed this is the case. In *C. elegans*, *Drosophila* and mice, down-regulation of the TOR pathway prolongs lifespan (Johnson et al., 2013). The prevailing view of the AMPK/TOR interaction is that AMPK acts primarily as an upstream suppressor of TOR. AMPK inhibits TOR signaling by two means: first, AMPK phosphorylates the tuberous sclerosis protein 2 (TSC2), which is an upstream inhibitor of TORC1 (Inoki et al., 2003). Second, it phosphorylates raptor to directly inhibit TORC1 (Gwinn et al., 2008). However, recent work suggests AMPK also lies downstream of TORC1. The TORC1 target S6 Kinase (S6K) phosphorylates AMPK α2 at serine 491. AMPK α2 Ser491 phosphorylation inhibits subsequent phosphorylation at Thr172 (Dagon et al., 2012), thus suppression of TORC1 can activate AMPK. Possibly confirming this point, AMPK α/AAK-2 is required for the longevity of *C. elegans rsks-1*/S6K mutants (Selman et al., 2009). Therefore, despite the widely held view that AMPK and TOR function together to mediate lifespan, the exact mechanisms by which they exert their influences on longevity, and which one is downstream of the other, remains unclear. At present, the majority of work on the effects of AMPK on aging comes from *C. elegans*, in which a TSC ortholog has yet to be identified, and where there remains no evidence that AMPK and TOR are in fact co-regulated.

FOXOs

Of the multiple nutrient and energy-sensing pathways recently identified to promote healthy aging, rlIS is the most potent, robust, and least controversial (Kenyon, 2010). rl IS substantially increases longevity and prolongs healthy aging in multiple organisms, including mammals (Kenyon, 2010). In *C. elegans* and *Drosophila*, the sole FOXO transcription factor family member, is activated by rl IS and completely required for rl IS mediated longevity (Kenyon et al., 1993; Slack et al., 2011; Yamamoto and Tatar, 2011). Highlighting the translational potential of this pathway, single nucleotide polymorphisms in FOXO family members are linked to extreme longevity in multiple human populations (Kenyon, 2010). In *C. elegans, aak-2* is required for the longevity effects of rl IS (Apfeld et al., 2004). Suggesting that AMPK might activate FOXO, induction of the stress-resistant dauer larval stage seen in *C. elegans* with rllS requires FOXO and is suppressed in animals lacking *aak-2* (Apfeld et al., 2004). Furthermore, FOXO/DAF-16 is required for lifespan extension via activating mutations in the AMPK γ subunit in *C. elegans* (Greer et al., 2007a).

AMPK can directly phosphorylate both *C. elegans* (Greer et al., 2007a) and mammalian (Greer et al., 2007b) FOXO family members. However, it is not yet known if this event is causally or casually linked to lifespan extension. An alternative explanation for the requirement of FOXO for AMPK longevity is that FOXO acts upstream of AMPK or in a feedback loop. Indeed, DAF-16 has been shown to regulate expression of multiple AMPK components in *C. elegans* (Schuster et al., 2010; Tullet, 2014). Reduced *daf-16* may therefore deplete AMPK levels or alter the composition of the heterotrimer. Although AMPK can directly phosphorylate FOXO/DAF-16, it remains to be tested if this interaction is required for AMPK longevity.

Sirtuins

AMPK is an appealing link between energetics and longevity because it is directly activated by shifts in nucleotide balance induced by low energy. However, ATP is not the only 'fuel' whose levels deplete under low energy. Reduced metabolic flux also shifts balance toward a higher NAD^+:NADH ratio. One outcome of this change is the activation of a class of deacetylases known as the sirtuins, which require NAD for their enzymatic activity and are heavily implicated in aging (Haigis and Sinclair, 2010). Initial studies on the role of Sir2 and its orthologs in promoting aging were hampered by confounding effects of genetic background, enzymatic activity assays, and whether or not SirT1 is required for all effects of DR (Lombard et al., 2011). However, subsequent data demonstrate that activating Sir2 orthologs increases lifespan in specific yeast strains, worms, flies, and mice in the absence of concerns over background effect (Guarente, 2013). Despite their problems, excitement over the initial invertebrate experiments spawned multiple experiments testing the effect of sirtuins on mouse, and here the breadth of data shows without doubt that these critical metabolic regulators have a role in age-related pathology and might be targeted to promote healthy aging (Guarente, 2013).

AMPK and sirtuins are therefore both critical fuel sensors with positive effects on longevity, and there is substantial cross talk between them (Ruderman et al., 2010). Indeed, lifespan extension in *C. elegans* strains overexpressing the worm sirtuin, *sir-2.1*, require *aak-2* (Curtis et al., 2006), although these experiments were done in lines harboring a mutation at an additional locus, which may be confounding (Burnett et al., 2011). AMPK can activate SirT1 by altering the NAD:NADH ratio (Canto et al., 2009; Fulco et al., 2008) and this interaction is critical for maintaining energy homeostasis. Fasting induces a switch in fuel usage from glucose to lipid in skeletal muscle mediated in part by SirT1 promoting mitochondrial biogenesis and gene expression via PGC-1 alpha in an AMPK dependent manner (Canto et al., 2010). AMPK also acts downstream of SirT1, as mice lacking SirT1 show blunted activation of AMPK by either Resveratrol or AICAR administration (Price et al., 2012). SirT1 deacetylates and activates LKB1, leading to increased phosphorylation of AMPK α Thr172 (Lan et al., 2008). Therefore, as is the case for mTOR, AMPK and SirT1 co-regulate each other, and how they coordinate to mediate lifespan requires further evaluation.

CRTCs

Recently cAMP-responsive element-binding protein (CREB)-regulated transcriptional coactivators (CRTCs) were shown to link AMPK activity to the transcriptional regulation of lifespan (Mair et al., 2011). CRTCs are transcriptional cofactors involved in diverse physiological processes including energy homeostasis, mitochondrial biogenesis, and ER stress (Altarejos and Montminy, 2011). CRTCs are direct targets of AMPK and their aberrant regulation is causal to multiple complex diseases (Altarejos and Montminy, 2011). CRTCs were first identified as CREB coactivators capable of inducing expression of CREB targets by facilitating recruitment of the transcriptional apparatus (Conkright et al., 2003; Iourgenko et al., 2003); but CRTCs also interact with bZIP transcription factors such as AP-1 and ATF-6 (Canettieri et al., 2009; Wang et al., 2009b). CRTCs are negatively

regulated through phosphorylation by AMPK-family kinases, which facilitates 14-3-3 protein binding and retention of CRTCs in the cytoplasm (Screaton et al., 2004).

We investigated whether CRTCs played a role in mediating the longevity effects of AMPK in *C. elegans*. There is a single *C. elegans* CRTC family member, CRTC-1, which is expressed in the nervous system and the intestine. Along with digestive function, the intestine is thought to play liver- and adipose-like roles in the worm. CRTC-1 is inactivated by low nutrients and AMPK (Mair et al., 2011). Mutating two conserved AMPK target serines on CRTC-1 (S76 and S179) to alanine eliminates its regulation by AMPK, resulting in constitutively nuclear and active CRTC-1. Constitutively active (CA) CRTC-1S76A, S179A completely suppresses AMPK-mediated longevity.

Although the link between AMPK and CRTCs is tightly conserved in *Drosophila* (Choi et al., 2011) and mammals (Altarejos and Montminy, 2011), its effects on lifespan are as yet untested in these models. Mammals have three CRTCs expressed predominantly in brain (CRTC1), liver (CRTC2) and white adipose tissue (CRTC3). Each CRTC isoform can have separable roles in distinct tissue types. For example, CRTC2 regulates energy homeostasis in the liver (Koo et al., 2005) and acts as an energy sensor in the hypothalamus (Lerner et al., 2009), the area of the brain responsible for nutrient sensing. Determining which tissue is responsible for the effect of CRTC-1 on longevity will therefore provide valuable insight into the mechanism of CRTC-1 mediated longevity, along with which mammalian CRTC might be playing an analogous role.

Additional Longevity Mediators and AMPK

To date, TOR, rllS/FOXO, Sirtuins and CRTCs are the pathways most directly implicated in lifespan extension by AMPK. However, AMPK activity is associated with several additional known longevity mediators, though causal association has yet to be determined. For example, perturbed mitochondrial function extends lifespan in worms and flies, and in *C. elegans* this effect is blunted in *aak-2* mutants (Curtis et al., 2006). A further link between mitochondrial perturbations, AMPK, and aging comes from work on ‘I'm not dead yet’ (INDY), which encodes a transporter for Kreb cycle intermediates. Reduced *indy* expression increases lifespan in *C. elegans* and *Drosophila* (Wang et al., 2009a) and mimics the transcriptional effects of DR in mice (Birkenfeld et al., 2011). Interestingly, INDY −/− mice are protected from HFD induced obesity, have increased AMPK activity, and increased hepatic mitochondrial density. Suggesting a causal role for AMPK in the beneficial effects of INDY mice, the AMPK inhibitor compound C has no effect on lipid synthesis in control mice, yet increases FA synthesis of INDY −/− mice by 240%. An additional link between AMPK and aging is the hypoxia inducible factor, HIF-1. Stabilization of HIF-1 in *C. elegans* extends lifespan (Mehta et al., 2009), and in human prostate carcinoma cells, AMPK is required for HIF-1 activation by hypoxia (Lee et al., 2003). However, lifespan extension under hypoxic conditions does not require AAK-2 in *C. elegans* (Leiser et al., 2013), and as yet a causal role of AMPK in HIF-1 mediated longevity has not been tested. Importantly, the catalogue of AMPK substrates is unlikely to be complete. The kinase's reach has recently expanded into areas of biology where connections to aging or longevity remain

underexplored, including for example epigenetics (Bungard et al., 2010; Mihaylova et al., 2011), Circadian rhythm (Lamia et al., 2009), and mitosis (Banko et al., 2011).

AMPK and Aging Processes

Aging does not result from the failure of a single homeostatic mechanism; therefore an ideal target for promoting longevity will have widespread outcomes that globally promote cellular homeostasis and repair. Here we examine the effect of AMPK on a variety of processes known to impact aging (Figure 3). We highlight the gap between biochemical insights gleaned from mammalian systems and the genetic and lifespan studies performed in invertebrates.

Autophagy

Autophagy has emerged as a core process for longevity assurance (reviewed in depth by Rubinsztein et al., 2011). Macroautophagy (henceforth ‘autophagy’) is a highly conserved process whereby a membrane-bound vesicle, the autophagosome, engulfs cytoplasmic contents and delivers the enclosed intracellular macromolecules and/or organelles to lysosomes for degradation. The end result is a recycling of cellular components to produce a fresh pool of intermediate compounds to accommodate the biosynthetic and energetic demands of the cell. Autophagy also promotes molecular and organellar quality control by degrading damaged or misfolded proteins and even damaged mitochondria in a process termed mitophagy. Most longevity-promoting interventions require an intact autophagic machinery; furthermore, reduced autophagic activity is associated with aging while evidence suggests that enhanced autophagy promotes longevity and delays age-related phenotypes (Rubinsztein et al., 2011).

Since autophagy can mobilize pre-existing cellular resources, it becomes especially important when nutrients are scarce. As such, DR is a potent inducer of autophagy and AMPK plays an important role in this process (Rubinsztein et al., 2011). AMPK phosphorylates and activates UNC-51-like kinase 1 (ULK1), a conserved component of the autophagy initiation complex (Egan et al., 2011; Kim et al., 2011a). ULK1 is required for the formation of the autophagosome, and mammalian cells lacking ULK1 display deficient basal mitophagy and sensitivity to starvation (Egan et al., 2011). AMPK also regulates Vps34, a conserved phosphatidylinositol 3-kinase (PI3K) that plays diverse roles in the cell depending on the protein complex in which it resides. In response to autophagy-inducing conditions or pharmacological activation, AMPK differentially phosphorylates functionally distinct Vps34 complexes, such that pro-autophagy Vps34/Beclin1/Atg14 complexes are activated by AMPK phosphorylation of Beclinl, while complexes without a role in autophagy are inhibited by AMPK phosphorylation of Vps34 itself (Kim et al., 2013).

AMPK may also regulate autophagy through interactions with other longevity pathways. For example, rlIS is known to enhance autophagy at least in some tissue types in worms, flies, and mammals (Demontis and Perrimon, 2010; Melendez et al., 2003), but whether FOXOs downstream of rl IS require a priming phosphorylation by AMPK to mediate autophagic responses is unknown (Greer et al., 2007b; Mammucari et al., 2007). SirT1 directly deacetylates multiple components of the autophagic machinery, and mice lacking SirT1

have deficits in basal autophagy and AMPK activation, and die shortly after birth (Lee et al., 2008). Several recent studies highlight autophagy as a central node for AMPK-TOR signal integration. mTOR phosphorylates ULK1 at a site closely neighboring AMPK phosphorylation to inhibit the AMPK-ULK1 interaction and prevent activation of autophagy (Kim et al., 2011a). Further, mTOR phosphorylates and cytoplasmically sequesters the transcription factor EB (TFEB), a master regulator of autophagic and lysosomal genes, thereby inhibiting autophagy at the level of gene expression (Settembre et al., 2011; Settembre et al., 2012). The nematode ortholog of TFEB, HLH-30, is required for multiple pro-longevity interventions, and upon over-expression is sufficient for lifespan extension (Lapierre et al., 2013; O'Rourke and Ruvkun, 2013). Whether AMPK directly regulates TFEB/HLH-30 is unknown, but it is possible AMPK enhances TFEB activity through its suppressive effects on TOR. Lastly, transgenic activation of AMPK and inhibition of calcineurin both extend longevity in *C. elegans* by converging to regulate the activity of CRTC-1 (Mair et al., 2011), and calcineurin longevity requires the autophagic pathway (Dwivedi et al., 2009). While it has not yet been determined whether longevity induced by AMPK requires the autophagic machinery, it is tempting to speculate that CRTC orthologs may play a role downstream of AMPK in the transcriptional regulation of autophagy.

AMPK thus communicates with numerous pathways and proteins to coordinate autophagy with cell and environmental conditions, but more focused mechanistic studies are needed to determine which mode of regulation, if any, plays a dominant role in the context of aging. Invertebrate genetic analyses tell us that numerous longevity pathways require intact autophagic machinery, but dissecting which regulatory routes are important for healthy aging remains difficult until the mostly mammalian-defined signaling mechanisms delineated above have been confirmed in worms or flies. As an example, Egan et al. (2011) demonstrated that nematodes expressing a constitutively active AMPKα display greater autophagosome numbers in an ULK1-dependent manner, but both the biochemical interaction and the requirement of this genetic interaction for AMPK-mediated longevity remain unconfirmed. Demonstrating the promise of targeting autophagy in mammals, overexpressing a key protein in expansion of autophagosome membranes in mice enhances autophagy, maintains metabolic fitness later in life, and extends lifespan ~17% (Pyo et al., 2013).

Mitochondrial Biogenesis

Mitochondrial homeostasis is linked to multiple pathways known to modulate aging, and AMPK plays vital roles as both a sensor and regulator of mitochondrial function. Mitochondrial activity declines with age while production of potentially toxic reactive oxidative species (ROS) increases, in part through the impaired ability of AMPK to generate new mitochondria in aged organisms (Reznick et al., 2007). Although early hypotheses linking mitochondria to aging focused on ROS damage, we now appreciate mitochondria play diverse roles in processes as disparate as apoptosis, intermediary metabolism, and calcium homeostasis, and there is growing appreciation that ROS can play critical signaling roles. Impairments of glucose metabolism and rllS, both of which require AMPK to increase lifespan (Apfeld et al., 2004; Schulz et al., 2007), induce a transient increase in the AMP/ATP ratio and trigger a burst of ROS production in nematodes (Zarse et al., 2012).

This ROS signal promotes longevity by signaling a lasting shift in mitochondrial function to increase respiration and enhance antioxidant defense capacity, a process termed "mitohormesis", which requires *aak-2*/AMPKα (Schulz et al., 2007).

Despite recent progress linking mitochondrial pathways to aging, how AMPK mediates crosstalk between mitochondrial functioning and longevity-related pathways remains incompletely understood, especially in worms and flies. In mammals AMPK is capable of both acute and long-term modulation of mitochondrial activity, controlling substrate selection for mitochondrial fuel. In response to fasting and exercise, myocytes switch from glucose to fat oxidation for energy, and AMPK is the driving force for that switch (Canto et al., 2010). AMPK also remodels transcriptional networks to control mitochondrial biogenesis and turnover via mitophagy. Loss of two AMPK-β units in murine muscle reduces mitochondrial activity and greatly diminishes muscle performance (O'Neill et al., 2011). Conversely, activation of AMPK by AICAR (Winder et al., 2000) or energy deprivation induced by guanidinopropionic acid (Zong et al., 2002) increases mitochondrial gene expression and activity, and a 4-week treatment of AICAR confers the benefits of endurance training to "sedentary" mice in terms of both muscle mitochondrial profiles and actual treadmill endurance (Narkar et al., 2008). The critical transcriptional effector of AMPK-mediated mitochondrial biogenesis appears to be peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC1α), a direct target of AMPK phosphorylation (Jager et al., 2007). AMPK also promotes SirT1 activity by increasing the cellular concentration of its cofactor, NAD^+, and SirT1 subsequently deacetylates PGC1α (Canto et al., 2009). Interestingly, enhanced mitochondrial homeostasis mediated by overexpression of *Drosophila* PGC1α is sufficient to extend lifespan in flies; and while ubiquitous PGC1α overexpression shortens lifespan, enhancing PGC1α specifically in intestinal stem cells improves tissue maintenance and promotes longevity (Rera et al., 2011). To date, however, AMPK has not been linked to PGC1α in flies and no PGC1α ortholog has been identified in *C. elegans*. Taken together, we know that AMPK senses and regulates mitochondrial homeostasis in mammalian systems and that AMPK activity is sufficient to promote lifespan in lower organisms. Importantly, however, there is surprisingly little evidence that AMPK directly modulates mitochondrial function in worms or flies, and thus it is too early to conclude that AMPK promotes longevity through a mechanism involving altered mitochondrial function.

Lipid Metabolism

Several lines of evidence link longevity downstream of rllS, TOR, and autophagy to global alterations in fat metabolism or lipid-derived signaling pathways (Lapierre et al., 2011; O'Rourke and Ruvkun, 2013; Soukas et al., 2009; Wang et al., 2008). Surprisingly, despite AMPK's involvement in these longevity pathways and its long-established roles regulating lipid metabolism, it remains unknown whether AMPK links lipid metabolism and longevity. Indeed, despite the accepted paradigm that fat suppresses metabolic homeostasis in mammals, paradoxically, in lower organisms, longevity-promoting interventions such as MIS and DR can increase lipid deposition and accumulation (Hansen et al., 2013). Current data suggest differences in lipid composition (i.e., saturation, length) rather than amount might be more critical in modulating the rate of aging. Supporting this concept, long-lived

germline-less worms activate a conserved pathway involving a nuclear hormone receptor, NHR-80, and subsequent enhancement of stearate desaturation to oleate via a stearoyl-CoA desaturase (SCD) gene *fat-6*/SCD1 (Goudeau et al., 2011). Similarly, overexpression of the lipase LIPL-4 in *C. elegans* confers longevity by generating higher concentrations of ω-6 fatty acids, which subsequently enhance autophagy (Lapierre et al., 2012; O'Rourke et al., 2013; Wang et al., 2008). Therefore, an important factor in aging may not be altered adiposity per se, but secondary intra- or intercellular lipokine signaling events mediated by lipid intermediates, and fat mobilization between tissues. In *Drosophila*, DR enhances both triglyceride synthesis and triglyceride catabolism, and this apparent 'cycling' of fat involves synthesis and mobilization of triglyceride between the fat body and the muscle, where it is catabolized to support enhanced tissue function (Katewa et al., 2012). Intriguingly, inhibition of fat utilization in the muscle abrogates the lifespan effects of DR, suggesting the role of fat metabolism in aging involves complex coordination of both lipid signaling and mobilization. Consistent with this idea, genetically arrested germline stem cells in worms send signal(s) to the intestine to activate lipolysis, and overexpression of the lipase responsible, LIPL-4, is sufficient to promote longevity (Wang et al., 2008). While the growing number of links between fat metabolism and aging are exciting, there is still much to learn about the dynamics of fat utilization and signaling.

As a core regulator of fat metabolism, AMPK is an important piece in this puzzle, but investigations into whether AMPK mediates its effects on aging through lipids are only now beginning. AMPK inhibits the 'master regulator' of lipogenesis, sterol regulatory element binding protein (SREBPc) via direct phosphorylation (Li et al., 2011). In mammals AMPK also acutely alters the fate of intracellular lipids, promoting beta-oxidation and suppressing lipogenesis and sterol synthesis through targets such as acetyl-CoA carboxylase (ACC) 1 and ACC2, fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), and glycerol-3-phosphate acyltransferase (GPAT) in various tissues (Hardie et al., 2012), but many of these regulatory links remain untested in invertebrate longevity models. A small molecule screen for compounds with effects on fat levels in *C. elegans* identified a novel agonist selective for *aak-1* (Lemieux et al., 2011). The molecule also activated AMPK signaling in mammalian cells, demonstrating both the utility of the worm for screens involving organism-wide physiology and a conserved role for AMPK in the global regulation of fat metabolism. Intriguingly, *C. elegans* AMPK-α/AAK-2 mutants, which might be predicted to have suppressed lipid mobilization, burn through fat stores faster than wild type animals under conditions of starvation, suggesting that AMPK rations fat reserves over the long-term and may be required for maintaining the levels of certain lipid species (Narbonne and Roy, 2009). This general function, if not the precise mechanism, seems conserved in flies (Braco et al., 2012). Whether this role in fat rationing occurs to any extent in mammals is unclear, but AMPK activation in the adipose tissue can inhibit hormone sensitive lipase (HSL), and thus potentially slow down lipolysis (Daval et al., 2005). Generally, many mechanistic links between AMPK and lipid metabolism remain unconfirmed in worms and flies, and future studies probing AMPK function in fat metabolism and aging will need to address both its effect on lipid signaling alongside its role as a cell-autonomous regulator of lipid utilization.

Central Control of Energy Homeostasis

Regulation of longevity by the central nervous system has emerged from studies in worms, flies, and rodents (Alcedo et al., 2013). Intriguingly, many of the neuronal subtypes associated with these longevity-related functions also sense or convey changes in energy homeostasis across tissues. Consistent with AMPKs energy-sensing function at the cellular level, hormonal orexigenic signals indicating low energy in peripheral tissues, e.g., ghrelin, activate AMPK in the mammalian hypothalamus, while anorexigenic signals indicating neutral or high energy status, i.e., leptin, insulin, and high glucose, have the opposite effect (Minokoshi et al., 2004). AMPK activation in this energy-sensing region of the brain is sufficient to increase food intake and body weight, thus restoring energy balance to the organism (Minokoshi et al., 2004). However, endogenous signals do not activate AMPK universally across tissues. Leptin, for example, inhibits AMPK in the hypothalamus while activating it in muscle (Minokoshi et al., 2004; Minokoshi et al., 2002). In evaluating the potential for development of a potent pharmacological activator of AMPK to increase longevity, this particular role of the kinase presents an interesting dilemma: will activating AMPK in the hypothalamus cause hyperphagia, thus abrogating the beneficial metabolic effects stemming from the periphery?

While a central role for AMPK in sensing and responding to changes in energy homeostasis is conserved (Braco et al., 2012; Cunningham et al., 2012), the extent and mechanisms by which central AMPK activity drives specific metabolic processes in peripheral tissues remains mostly unexplored. In response to activation by ghrelin, AMPK metabolically potentiates the electrical hunger signal in NPY/AgRP neurons by stimulating mitochondrial fatty acid oxidation through carnitine palmitoyl transferase 1 (CPT1) and Uncoupling Protein 2 (UCP2)-dependent ROS scavenging (Stark et al., 2013). Intriguingly, ghrelin also stimulates CREB and FOXO transcription of the NPY and AgRP neuropeptides, respectively, and activation of these transcription factors requires CPT1 and UCP2, suggesting AMPK may be involved in this transcriptional pathway as well (Lage et al., 2010). The precise molecular mechanism connecting ghrelin and AMPK activity to CREB and FOXO remains undefined, giving cause to speculate on a potential role for CRTC orthologs in this pathway. Lerner et al. have described an AMPK-CRTC interaction in the murine hypothalamus whereby AMPK inhibits CRTC2 to reduce expression of insulin signaling components, e.g. Irs2 (Lerner et al., 2009). Future explorations in this relatively young area of neuronal AMPK biology may determine whether AMPK centrally and cell non-autonomously promotes longevity.

Stem Cells and Tissue Rejuvenation

Aged animals display functional deficiencies across diverse tissue types. In higher organisms tissue function is compromised as stem cell populations accumulate damage and eventually fail to replenish somatic cells lost during normal ‘wear and tear.’ This suggests that prolonging the capacity of stem cells to rejuvenate tissues might confer longevity. Validating this concept, longevity-promoting interventions including DR enhance stem cell maintenance across broad evolutionary distances (Cerletti et al., 2012; Mair et al., 2010). Though *C. elegans* lacks somatic stem cells, worms arrest development in the absence of food and coordinately halt proliferation of the germline, a process driven by asymmetric

division of germline stem cells (GSCs). Fukuyama et al. (2012) and Narbonne and Roy (2006) have demonstrated that AMPK is required for GSC quiescence in response to nutrient deprivation in L1 larvae or dauer larvae, respectively. Further, AMPK-deficient larvae that are fasted become sterile adults, suggesting AMPK is required for GSC function and/or viability after nutrient stress (Fukuyama et al., 2012).

In mammals, LKB1 is required to maintain quiescence in hematopoietic stem cells (HSCs) (Gan et al., 2010; Gurumurthy et al., 2010; Nakada et al., 2010). Mice with a conditional LKB1 knockout specifically in HSCs are short-lived and initially display increased proliferation followed by a pronounced depletion of HSCs. LKB1 loss also results in altered HSC mitochondrial function and reduced ATP levels. Surprisingly, the data suggest that neither AMPK nor mTOR is relevant to the observed effects on HSC proliferation. Loss of AMPK α1/α2 phenocopies only some mitochondrial deficits of the LKB1 null HSCs, but not the depletion in cell numbers, and though mTOR activity is enhanced, rapamycin also fails to rescue the effects on HSC reconstitution (Nakada et al., 2010). More work is therefore required to define the role of AMPK in mammalian stem cells and tissue rejuvenation.

Protein Synthesis

Reductions in protein synthesis by knockdown of ribosomal proteins or interference with translation initiation factors confer longer lifespan across species (Kennedy and Kaeberlein, 2009). Reduced translation is also associated with longevity mediated by both DR and TOR inhibition (Bjedov et al., 2010; Hansen et al., 2007; Zid et al., 2009), and AMPK can reduce protein synthesis by one of several mechanisms. Activated by anoxia or pharmacologically, AMPK acutely represses protein synthesis by phosphorylating the upstream inhibitory kinase of eukaryotic elongation factor 2 (eEF2) (Horman et al., 2002). AMPK also regulates protein synthesis indirectly through its crosstalk with the TOR pathway. Metformin treatment of cancer cells leads to AMPK-dependent inhibition of mTOR and its downstream targets, S6K and 4E-BP, to reduce protein synthesis by ~30% at the point of translation initiation (Dowling et al., 2007). AMPK also suppresses global protein biosynthetic capacity by reducing rRNA production (Hoppe et al., 2009). Consistent with the tendency for long-term AMPK effects to occur at the level of gene expression, this mechanism involves AMPK mediated phosphorylation and inhibition of TIF-IA, an essential initiation factor for RNA polymerase I.

Despite these connections between reduced protein synthesis, AMPK, and the DR or TOR longevity pathways, it is unclear whether AMPK requires any of its effects on the translational machinery to prolong lifespan. Further, though reduced protein synthesis robustly extends lifespan across models, including nematodes, which lack a clear 4E-BP ortholog, the mechanism downstream of protein synthesis itself remains to be determined. A recent study suggests that the beneficial effects may stem from preferential synthesis of stress protective proteins under these conditions, as opposed to a decrease in total protein production or concentration *per se* (Rogers et al., 2011). Future application of techniques such as polysome profiling or proteomic analyses to systems involving enhanced AMPK activity may be informative in this regard.

AMPK and Aging Pathologies

Given its broad role in maintaining cellular and organismal energy homeostasis, it is perhaps unsurprising that AMPK has been implicated in multiple pathologies, including many age-onset diseases reviewed extensively by Steinberg and Kemp (2009). However, as outlined below, the concept of AMPK being universally protective in age-related pathologies is over-simplistic. In the case of key age-onset diseases such as cancer and neurodegenerative diseases, AMPK may even be pathogenic. Here we discuss examples where AMPK can be both protective and harmful for the same pathology, and as such, how context is critical when thinking about targeting AMPK therapeutically.

Cancer

Cancer cells undergo dramatic shifts in metabolism and override checkpoints meant to coordinate growth with energy supply. Perhaps unsurprisingly, AMPK deregulation has been implicated in tumorigenesis. Type II diabetic patients taking metformin show protection against cancer, independent of metformin's effects on diabetes (Gallagher and Leroith, 2011). Metformin also inhibits cellular transformation (Hirsch et al., 2013) and delays p53 −/− tumor xenograft progression in mice (Buzzai et al., 2007). Furthermore, mutations to LKB1 are commonly associated with sporadic non-small cell lung carcinoma and causally linked to the inherited Peutz-Jeghers syndrome, which greatly increases cancer risk. Additionally, multiple loss-of-function LKB1 mouse models show a variety of cancer predispositions (Shackelford and Shaw, 2009). Whether these effects specifically result from AMPK inhibition, however, remains unclear. LKB1 phosphorylates and activates a family of 14 AMPK-like kinases, and redundancy between them may explain the infrequency of cancer-associated mutations in LKB1-dependent kinases, including AMPK. Indeed, whether AMPK acts as a tumor suppressor or in fact might be oncogenic is still uncertain (Liang and Mills, 2013).

Recent work suggests that AMPK is a negative regulator of the ‘Warburg effect’, a classical metabolic shift in cancer cells from oxidative phosphorylation towards increased aerobic glycolysis and lactate production, driving biomass production and growth. AMPK α1 deficient mice over-expressing the oncogene c-Myc in B-lymphocytes have increased lymphomagenesis, with tumors having increased glucose consumption and lactate production (Faubert et al., 2013). Paradoxically, however, AMPK can also support the metabolic changes needed to support tumor growth. Enduring energy stress and hypoxia, cancer cells are effectively “living on the edge,” and AMPK affords a metabolic plasticity that allows them to survive (Jeon et al., 2012; Liu et al., 2012). As such, under certain conditions AMPK is—if not strictly oncogenic—then at least onco-‘enabling.’ These data may explain why expression of AMPK components is often increased in human cancers (Liang and Mills, 2013). Therefore, despite encouraging data such as the tumor suppressive effects of the direct AMPK agonist A-769662 (Huang et al., 2008), more work is needed to differentiate between contexts where AMPK activation is therapeutic from those where it may be detrimental.

Inflammation and Metabolic Disease

Metabolic disease is an age-associated pathology by which deregulated glucose homeostasis leads to hyperglycemia, glucose intolerance, and insulin resistance. Given its role in maintaining energy balance, AMPK has long been touted as a potential therapeutic avenue, and metformin is one of the most widely prescribed treatments for type II diabetes. Although the extent to which AMPK mediates the beneficial effects of metformin on metabolic disease remains unclear (Foretz et al., 2010; Miller et al., 2013), there are multiple mechanisms by which this energy sensor can restore glucose homeostasis. As discussed above and reviewed comprehensively elsewhere (Hardie et al., 2012), the classical role of AMPK is promoting a shift from anabolic to catabolic processes, and thus activating AMPK might combat metabolic disease by suppressing synthesis of fatty acids, cholesterol, and triglycerides, while promoting fat burning via up-regulation of beta-oxidation and mitochondrial biogenesis. Increased and inappropriate glucose production is also associated with type II diabetes, and AMPK can suppress gluconeogenic genes through inhibition of CRTC/CREB targets (Koo et al., 2005). AMPK additionally stimulates glucose uptake into skeletal muscle by promoting translocation of the glucose transporter GLUT4 to the plasma membrane (Hardie et al., 2012). Together these data suggest activation of AMPK might be a promising target for metabolic disease and may underlie the striking protection of non-human primates on a DR regime against type II diabetes-like symptoms (Mattison et al., 2012).

In addition to impaired glucose homeostasis, recent work has pointed to a critical role for inflammation in metabolic disease (Hotamisligil, 2006). Interestingly, pro-inflammatory cells such as M1 macrophages and activated T cells undergo similar metabolic shifts seen in tumor cells, including increased glycolysis and flux through the pentose phosphate pathway. Conversely, anti-inflammatory cells such as regulatory T cells and M2 macrophages have reduced glycolysis, instead generating ATP via the TCA cycle and increased fatty-acid metabolism. AMPK dysfunction has therefore been suggested to be a causal link between obesity and inflammation, and a potential therapeutic target (O'Neill and Hardie, 2013). In support of this idea, anti-inflammatory cytokines activate AMPK a1 in macrophages while pro-inflammatory stimuli suppress it (Sag et al., 2008). Expression of a constitutively active form of AMPK also increases anti-inflammatory M2 phenotypes. In addition, mice with AMPK a1 deletion in macrophages have suppressed polarization of the anti-inflammatory M2 macrophages in skeletal muscle *in vivo* (Mounier et al., 2013). Taken together with its effects on the NF-κβ signaling (Salminen et al., 2011), AMPK is therefore implicated as a central link between obesity, inflammation, and metabolic disease, and is a potential therapeutic target.

Neurodegeneration

Deregulation of AMPK has been implicated in neurotoxicity-related diseases. AMPK is highly expressed in brain and protects against hippocampal neuron death during glucose deprivation (Culmsee et al., 2001). Additionally, resveratrol and AICAR increase AMPK phosphorylation and decrease Amyloid beta (Aβ) secretion from primary neurons in a mouse model of Alzheimer's disease (Vingtdeux et al., 2010), and this effect is blunted by the AMPK inhibitor Compound C. Resveratrol can be detected in the brain of APP mice fed the

compound, and these mice have reduced soluble and insoluble forms of Aβ (Vingtdeux et al., 2010). Genetic activation of AMPK also protects against neuronal loss in Parkinson's disease models in *Drosophila* (Ng et al., 2012). Conversely, inactivation of AMPK is linked to increased Alzheimer's risk in obese patients with type II diabetes (Kim et al., 2011b). In line with this model, palmitate, a free fatty acid associated with inflammation and obesity, suppresses AMPK and induces ER stress, tau phosphorylation, and apoptosis in human neuroblastoma cells, and this effect is blunted by AICAR and A-769662 (Kim et al., 2011b).

Much like tumorigenesis, however, AMPK's role as a ‘friend or foe’ in neurodegenerative processes remains uncertain. AMPK is often hyper-activated rather than suppressed in neurodegenerative diseases (Vingtdeux et al., 2011), and it remains unclear if this is a beneficial stress response or pathogenic. In the case of Alzheimer's, AMPK activation occurs via Aβ-induced increases in cellular calcium levels, which in turn stimulates the AMPK-kinase CaMKKβ (Thornton et al., 2011). Suggestive of a pathological role of AMPK in neurodegenerative disease, AMPK is a Tau kinase and as such may be the mechanistic link between Aβ and tau hyper-phosphorylation (Thornton et al., 2011). Metformin also increases Aβ levels in an AMPK dependent manner (Chen et al., 2009). AMPK α1 expression and nuclear localization is also increased in mouse models of Huntington's disease (HD) and brains of HD patients (Ju et al., 2011). In HD model R6/2 mice, this AMPK activation promotes neuronal loss and brain atrophy (Ju et al., 2011). It therefore remains to be determined if targeting AMPK will be useful therapeutically in neurodegenerative disorders, and if so, whether the goal should be activation or inhibition.

Future Directions - Translating Genetics of Aging to Therapeutics

Rather than targeting individual proximal causes of senescence and disease, research into the molecular genetics of longevity aims to beneficially impact a multitude of age-onset disorders with a single intervention. AMPK represents a promising therapeutic target for promoting healthy aging, as it sits upstream of multiple genetic pathways and homeostatic processes known to mediate longevity. However, AMPK's inter-connected nature perhaps limits its potential, since AMPK induces both beneficial effects on longevity and detrimental physiological effects such as growth inhibition and reduced reproductive capacity in invertebrates. Although activating AMPK may have therapeutic potential in diverse disease models, the effects remain very context-specific, and in the case of age-onset disorders such as cancer and neurodegeneration, AMPK may support disease progression.

At present the bulk of the biochemical and signaling data on AMPK has been generated using mammalian systems, with longevity data coming from invertebrates, where mechanisms of AMPK action are often assumed rather than demonstrated (Table 1). Defining how AMPK promotes longevity in lower organisms, and whether these effects are conserved in mammals will require merging the two disciplines of signaling and genetics. The conserved effects of AMPK on aging therefore represent just the beginning of a field, and exciting challenges lie ahead if we are to translate these data to useable therapeutics to promote healthy aging.

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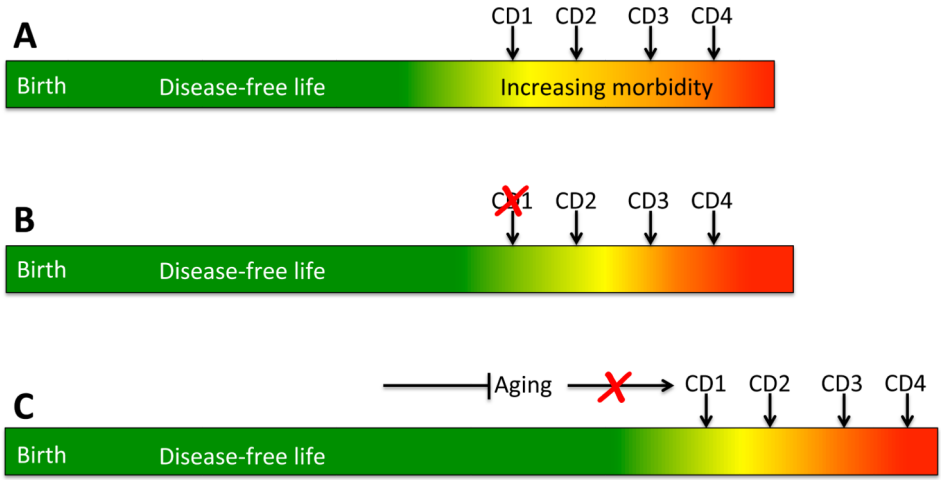

Figure 1. Co-morbidities and Age-related Pathologies

Each bar represents pathological state of an individual patient from birth to death. Green equals no pathology. As each chronic disease (CD) presents, decreases in health is portrayed by increased 'heat' of color. **A**. Patients over 65 rarely have one age-related pathology in isolation, but instead have multiple comorbidities that develop concurrently. **B**. Therefore, eradicating one disease (CD1) in it entirety will not dramatically increase disease free life. **C**. Targeting age-related frailty and systemic disease risk might alternatively delay the onset of multiple pathologies and greatly improve healthy aging.

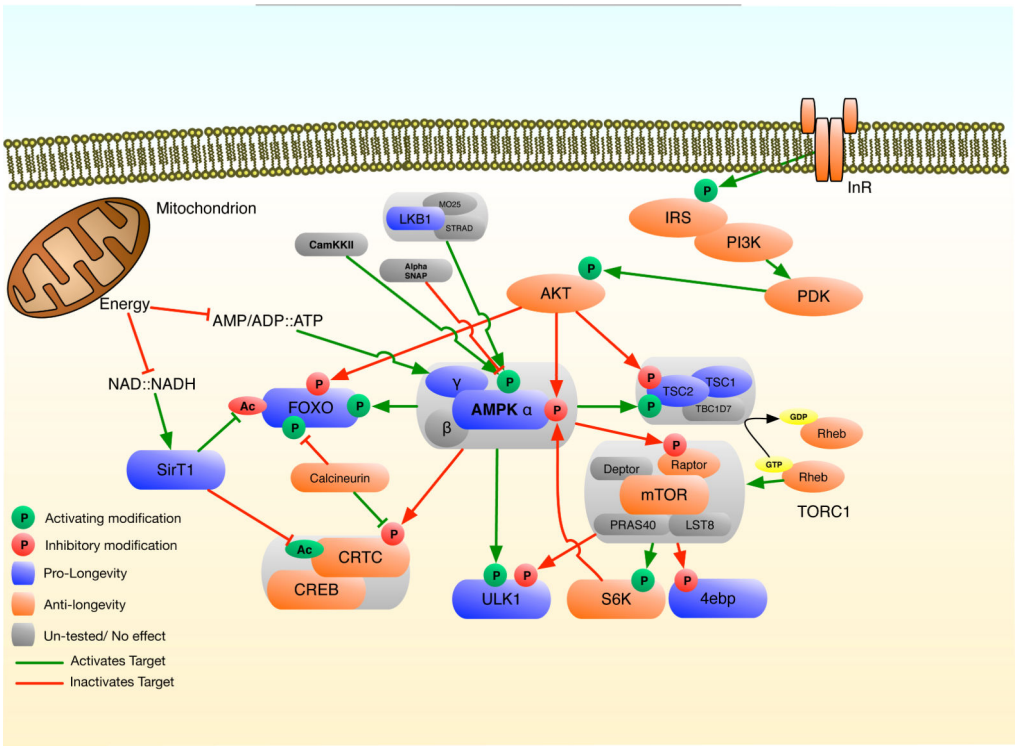

Figure 2. AMPK Signaling and Aging Pathways

AMPK sits upstream of multiple signal transduction pathways known to modulate the aging process. Lines represent direct signaling effects. Color of line reflects effect on target – green equals activating, red equals inhibiting. Color of modification equals effect of modification – green equals activating, red equals inhibiting. Arrow signifies adds modification, T bar signifies removes modification. Therefore, for example, removing an inhibitory modification generates a green T bar, as the effect on the target is activation. Orange factors are anti-longevity, blue are pro-longevity.

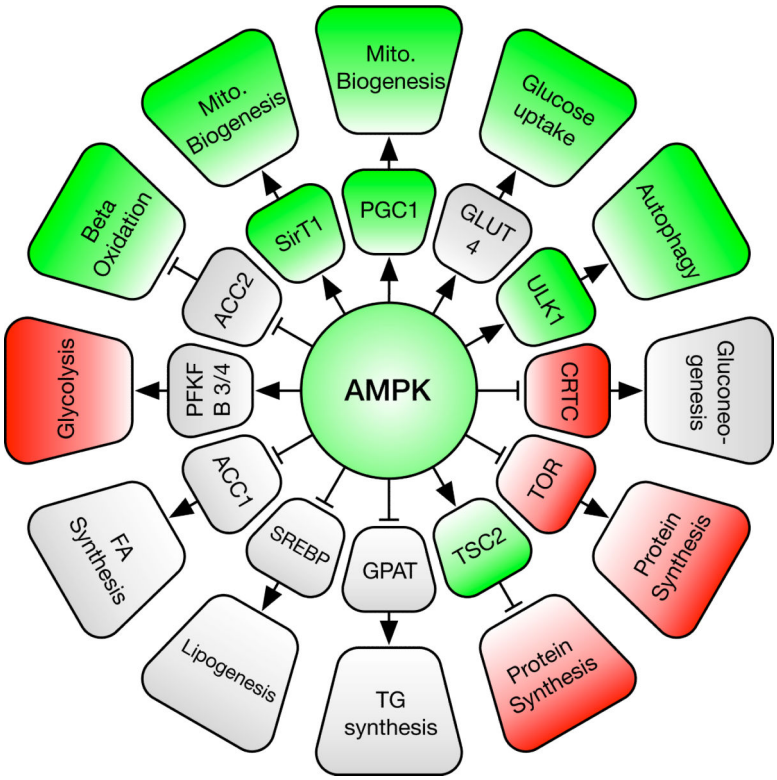

Figure 3. Physiological effects of AMPK

Schematic of selected physiological effects on AMPK and the intermediate mediators. Arrows signify activation, T bars signify suppression. Green signifies pro-longevity, red signifies anti-longevity.

Table 1

AMPK-related effects across model organisms. Check mark signifies data showing positive effect, cross signifies data showing no effect, question mark signifies effect unknown. Invertebrate ortholog names in parentheses.

Species	***C. elegans***	***Drosophila***	**Mouse**
Lifespan extension observed upon:			
AMPK activation	✓	✓	? ?
DR	✓	✓	✓
Metformin	✓	✘	✓
Resveratrol	✓ & ✘	✓ & ✘	✘
Aspirin	✓	✘	?
Direct AMPK Targets:			
FOXO	✓ (DAF-16)	? (FoxO)	✓
TSC2	✘ (None identified)	? (Tsc2)	✓
Raptor	? (DAF-15)	? (Raptor)	✓
CRTC	✓ (CRTC-1)	? (CRTC)	✓
ULK1	? (UNC-51)	? (Atg1)	✓
ACC1/2	? (POD-2)	✓ (ACC)	✓
SREBP	? (SBP-1)	? (HLH106)	✓
AMPK physiological effects observed upon:			
Mitochondrial Biogenesis	?	?	✓
Fatty Acid Oxidation	?	?	✓
Glycolysis	?	?	✓
Autophagy	✓	✓	✓
Activation of AMPK observed upon:			
DR	✓	?	✓ & ✘
Metformin/Phenformin	✓	✓	✓
Resveratrol	?	?	✓
Salicylate/ Aspirin	?	?	✓
Increased AMP:ATP	?	✓	✓