data would suggest that K_{ATP} channel-blocking drugs, which have been used for decades to treat type 2 diabetes, would stimulate β cell growth in patients, whereas the available data suggests that this is not the case. Clearly, sustained activation of glucokinase or blocking of K_{ATP} channels differs substantially from the intermittent stimulus provided by physiologic changes in glycemia. Furthermore, even if glucose metabolism is central for β cell growth, other factors may also be required to sustain its effects on long-term β-cell growth. This challenging question is worth solving, because decreased β cell mass is central to the pathophysiology of both type 1 and type 2 diabetes (Donath and Halban, 2004). Finding a safe compound that enhances β cell mass is therefore one of the holy grails of diabetes research. It is reasonable to assume that unraveling the sites of interaction of glucose metabolism with mitogenic pathways will provide more specific targets to develop such needed therapeutics. The findings reported here by Porat et al. (2011) are thus likely to spark considerable research in this area for years to come.

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A CRTCal Link between Energy and Life Span

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Cutting down calories prolongs life, but how this works remains largely unknown. A recent study in *Nature* (Mair et al., 2011) shows that life span extension triggered by the energy-sensing protein kinase AMPK is mediated by an evolutionarily conserved transcriptional circuit involving CRTC-1 and CREB.

Many of the proteins that control life span are also involved in nutrient sensing. One of these nutrient-sensing proteins, AMP-activated protein kinase (AMPK), is activated by a wide range of stimuli, including glucose deprivation, exercise, and the antidiabetic drug metformin (Steinberg and Kemp, 2009). AMPK is necessary for longevity induced by some, but not all, dietary restriction regimens in *C. elegans* (Greer et al., 2007; Greer and Brunet, 2009; Mair et al., 2009). Interestingly, overexpression of wild-type or active AMPK is sufficient to extend life span in worms (Apfeld et al., 2004; Greer et al., 2007). In mammals, AMPK has many reported substrates, including metabolism enzymes (acetyl CoA carboxylase), signaling molecules involved in the TOR pathway (RAPTOR, TSC2), autophagy regulators (ULK1), transcriptional coactivators (CRTC2), and transcription factors involved in the insulin pathway (FOXO3) (Egan et al., 2011; Steinberg and Kemp, 2009). While there is evidence for a role of the worm FOXO isoform in AMPK's ability to extend life span in *C. elegans* (Greer et al., 2007), exactly how AMPK mediates longevity has not yet been investigated in depth.

In their recent *Nature* study (Mair et al., 2011), Mair and colleagues identify a pathway connecting energy-sensing molecules to regulators of gene expression for life span regulation in *C. elegans*. Using an elegant combination of worm genetics, biochemistry, and immunocytochemistry, they delineate a conserved transcriptional network involving the transcriptional coactivator CRTC-1 and the transcription factor CREB in life span extension downstream of AMPK (Figure 1). Mair and colleagues initially show that CRTC-1 knockdown extends worm life span, similar to constitutively active AMPK expression or knockdown of calcineurin, a calcium-activated phosphatase whose attenuation extends life span. AMPK directly phosphorylates worm CRTC-1 at two conserved residues, thereby excluding CRTC-1 from the nucleus. A mutant of CRTC-1 that cannot be phosphorylated at AMPK sites is resistant to life span extension by AMPK and calcineurin modulations, suggesting that CRTC-1 phosphorylation is a major mediator of life span extension induced by AMPK activation and calcineurin inactivation.

How does CRTC-1 inactivation by AMPK extend life span? Similar to their

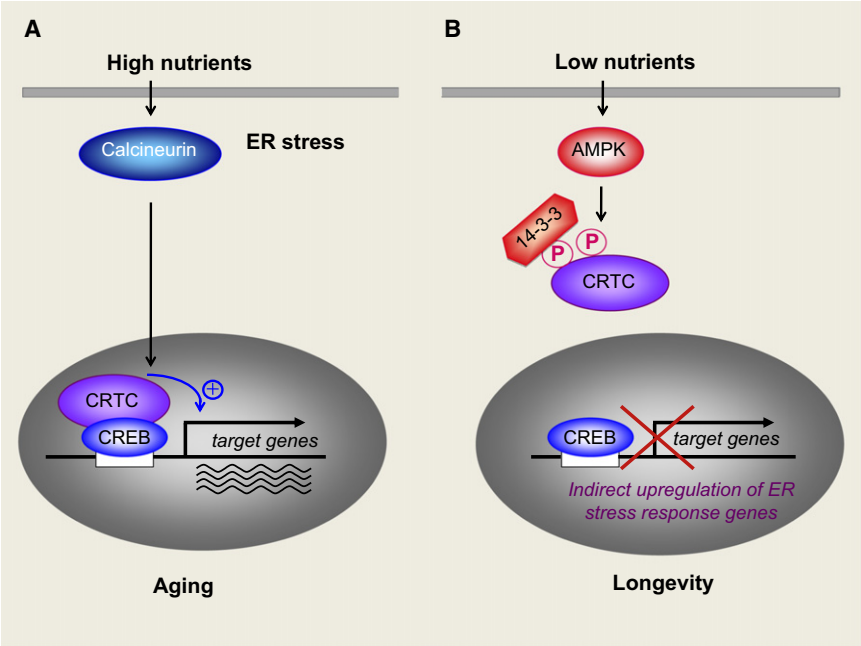

Figure 1. The CRTC-1/CREB Module Functions Downstream of AMPK and Calcineurin to Regulate Life Span in Worms

(A) In the presence of high nutrients, AMPK is inactivated whereas calcineurin is activated. CRTC-1 is dephosphorylated and translocates into the nucleus, where it binds to CREB to upregulate CREB-dependent target gene expression.

(B) Low nutrients activate AMPK. Activated AMPK directly phosphorylates CRTC-1 at two conserved sites, resulting in 14-3-3 binding and CRTC-1 sequestration in the cytoplasm. The inactivation of the CRTC-1/CREB module elicits the indirect upregulation of ER stress genes, which may contribute to life span extension.

mammalian counterparts, CRTC-1 and the transcription factor CREB physically interact in worms (Mair et al., 2011). Fittingly, CREB inactivation by knockdown also extends worm life span, although to a lesser degree than CRTC-1. Using a whole-genome microarray approach, the authors identify gene expression changes induced by this CREB network. They discover significant overlap between genes regulated by CREB and those regulated by AMPK and calcineurin, suggesting that CREB is a pivotal transcriptional target downstream of AMPK and calcineurin (Figure 1). Surprisingly, most of the genes regulated by AMPK, calcineurin, and CREB turned out not to be metabolic genes, but rather genes involved in endoplasmic reticulum (ER) stress and the unfolded protein response. A link between metabolic pathways and ER stress may be particularly important during aging, as unfolded proteins accumulate in older cells. It will be interesting to test which genes, among those regulated in common by AMPK, calcineurin, and CREB, are important for life span extension, and whether they contribute to longevity by modulating the unfolded protein response.

The study is exciting because it depicts an entire signaling pathway that connects an energy-sensing kinase and phosphatase, a transcriptional coactivator, a transcription factor, and downstream target genes to the regulation of life span. What is particularly remarkable is that this network and mechanisms underlying its regulation (e.g., CRTC phosphorylation and binding to the chaperone protein 14-3-3) are completely conserved in mammals (Screaton et al., 2004). While the mammalian CRTC ortholog and CREB are known to regulate fasting glucose metabolism downstream of AMPK in the liver (Altarejos and Montminy, 2011), their implication in longevity had never been assessed in mammals. The extreme conservation of this pathway raises the tantalizing possibility that manipulating this CRTC/CREB transcriptional network or target genes could impact longevity in mammals.

Several outstanding questions stem from this study. First, is the AMPK-CRTC-1/CREB network regulated by nutrients in worms, and does it contribute to dietary restriction-induced longevity? The CRTC-1/CREB transcriptional circuit may also be involved in the response to other types of stresses, including ER stress, as was shown in mammals (Altarejos and Montminy, 2011). Second, how does the calcineurin phosphatase fit with the AMPK-CRTC-1/CREB pathway? Calcineurin may directly dephosphorylate the AMPK-phosphorylated sites on worm CRTC-1, or calcineurin action on the CRTC-1/CREB network may be indirect. Third, in which tissues does the CRTC-1/CREB transcriptional network function to regulate life span? Mair and colleagues show that CRTC-1 is expressed in the same tissues as AMPK, suggesting a cell-autonomous role of the AMPK-CRTC-1/CREB module in longevity. Mammalian CRTC isoforms have been shown to play important roles in liver, brain, and fat (Altarejos and Montminy, 2011). Dissecting the specific tissues in which the AMPK-CRTC-1/CREB module is mostly required to regulate life span will provide pivotal insights into the mechanisms by which this network acts to promote longevity.

The findings described in this study are also interesting in the larger context of known substrates and functions of AMPK. Based on the genetic resistance of the nonphosphorylatable CRTC-1 to AMPK overexpression (Mair et al., 2011), CRTC-1 appears to be the exclusive direct substrate of both AMPK and calcineurin for life span regulation. Nevertheless, it will be important to determine whether and how the CRTC-1/CREB transcriptional circuit intersects with other substrates of AMPK, including FOXO transcription factors (Greer et al., 2007), ULK1 (Egan et al., 2011), and the ATGL1 lipase (Narbonne and Roy, 2009). Different AMPK substrates may belong to regulatory feedback loops or they may preferentially mediate AMPK's action in specific tissues. Conversely, it will be interesting to test if CRTC-1 and CREB are involved in additional biological roles of AMPK in worms, such as autophagy or dauer survival. More globally, which of the described functions of AMPK (tumor suppression, metabolic regulation, physiological response to exercise, etc.) are mediated by this

network versus other networks, and how are all these circuits integrated in a systematic manner? Finally, the connection between the energy-sensing kinase AMPK and the CREB transcriptional network is particularly intriguing in light of the known involvement of CREB in behavior and memory. Exploring how energy levels and ER stress affect CREB activity will be critical in understanding the balance between life span extension and the preservation of cognitive function.

This study further underscores that signaling and transcriptional modules that integrate information about nutrient status are critical for regulating life span and offers new points of entry for interventions that harness the longevity benefits of cutting down calories.

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