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Feature Review

Deregulation of CRTCs in Aging and Age-Related Disease Risk

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Advances in public health in the past century have seen a sharp increase in human life expectancy. With these changes have come an increased prevalence of age-related pathologies and health burdens in the elderly. Patient age is the biggest risk factor for multiple chronic conditions that often occur simultaneously within a single individual. An alternative to disease-centric therapeutic approaches is that of 'geroscience', which aims to define molecular mechanisms that link age to overall disease risk. One such mechanism is deregulation of CREB-regulated transcriptional coactivators (CRTCs). Initially identified for their role in modulating CREB transcription, the past 5 years has seen an expansion in knowledge of new cellular regulators and roles of CRTCs beyond CREB. CRTCs have been shown to modulate organismal aging in *Caenorhabditis elegans* and to impact on age-related diseases in humans. We discuss CRTC deregulation as a new driver of aging that integrates the link between age and disease risk.

Trends

Novel cellular regulators and targets of the CRTC family have recently been identified.

In *C. elegans* CRTCs have been shown to modulate aging.

Recently CRTC dysfunction has been associated with age-related human diseases.

CRTCs could provide a target for healthy human aging.

Aging as a Treatable Risk Factor

Public Health and Medical advances in the past century have yielded significant increases in human life expectancy. In 1955, worldwide average life expectancy was 46, but by 2015 this number had risen by nearly 20 years to 65 [1]. Because developing countries continue to get access to better healthcare, this trend is set to continue such that, by 2100, at least half of the human population worldwide can expect to live to 83 years of age [1] (Figure 1). The resulting shift in our population demographics means that, by 2050, there will be 1.5 billion people over the age of 65 [1] (Figure 1). Although this change is something to be lauded, our success in increasing our survival has come at a cost because, for the majority of us, these added years are not healthy. Age is the biggest risk factor for the majority of chronic diseases and, worse still, many of these conditions often occur simultaneously in the same individual – in the USA over half of people older than 65 years have two or more chronic conditions. This pandemic of comorbidities lessens the impact disease-centric medical approaches have on years spent free of disease; cure one disease completely and the patient is still left with the remaining ailments. An emerging alternative approach is that of 'geroscience' [2]. Instead of focusing on proximal mechanisms of individual age-related diseases in isolation, geroscience focuses on the commonality between all of them, namely aging itself, and on the cellular components that might link patient age to overall disease risk.

The past two decades have seen the discovery of genetic, environmental, and pharmacological perturbations that slow aging and decrease age-related pathology of model organisms. Although many of these interventions seem to function through non-overlapping mechanisms,

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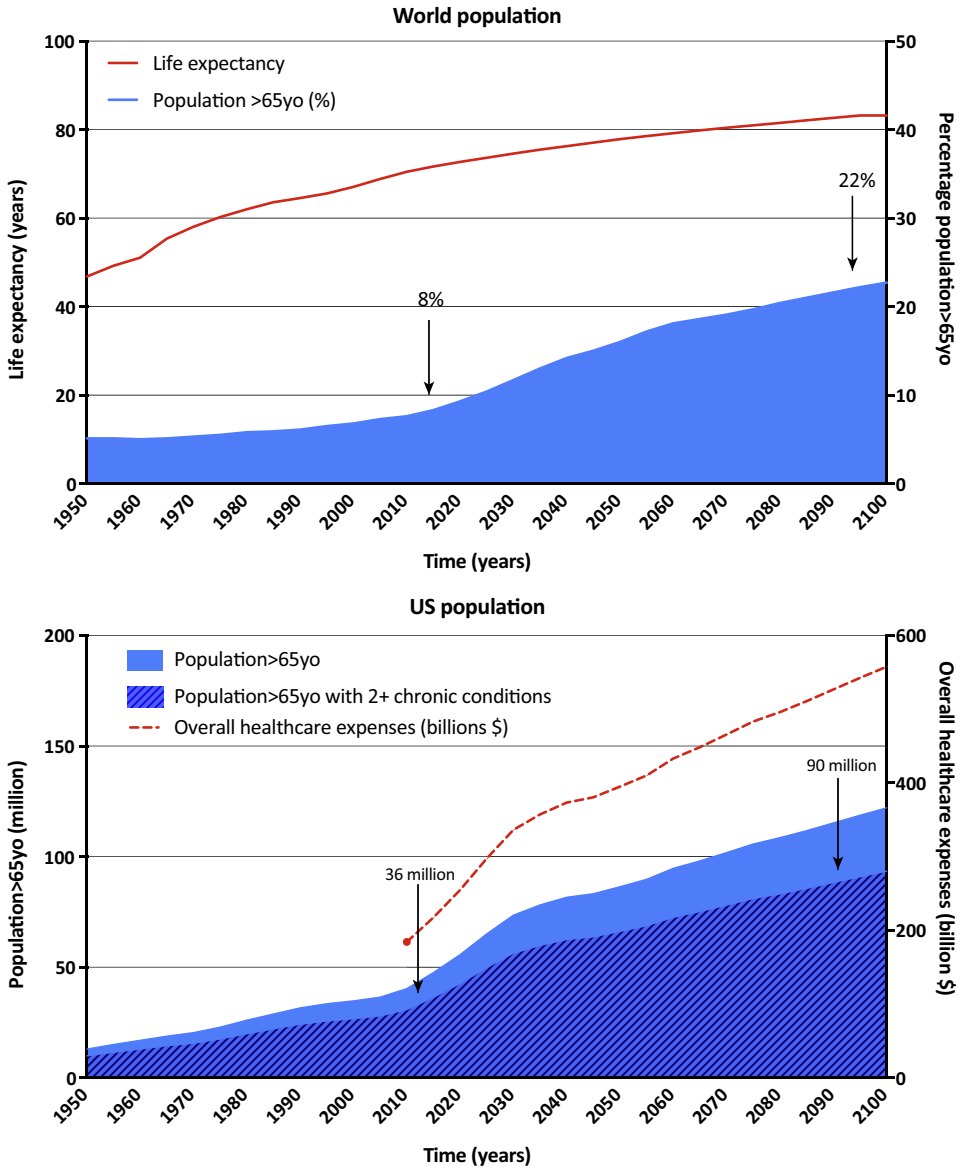


Figure 1. The Downsides of Increased Life Expectancy: An Aged Population Suffering from Multiple Chronic Diseases and Increased Economic Burden. (A) Increase in lifespan expectancy in the world from 1950 to 2015 and projected increase from 2015 to 2050. This increase is followed by an increase in the percentage of population aged >65 years (>65yo), representing 8%, and is predicted to represent up to 22% of the world population by 2050. (B) Proportional increase in people >65 years of age suffering from two or more (2+) chronic diseases as the population ages. This comorbidity will cause a subsequent increase in overall healthcare expenses (data extrapolated from expenses measured in 2015). Sources: United Nations [1]; Agency for Healthcare Research and Quality (AHRQ), https://meps.ahrq.gov/mepsweb/data_stats/publications.jsp; Centers For Medicare and Medicaid Services (CMS), 2012 Edition, https://www.cms.gov/research-statistics-data-and-systems/statistics-trends-and-reports/medicaremedicaidstatsupp/2012.html

one commonality is that lifespan extension is often coupled to reductions in energy-requiring processes and the rate at which energy stores are depleted. Decreasing food intake during dietary restriction (DR) increases longevity in species ranging from yeast to primates [3], and has protective effects against multiple age-onset conditions. Conserved mechanisms that integrate upstream energy-sensing pathways with targeted downstream transcriptional targets might

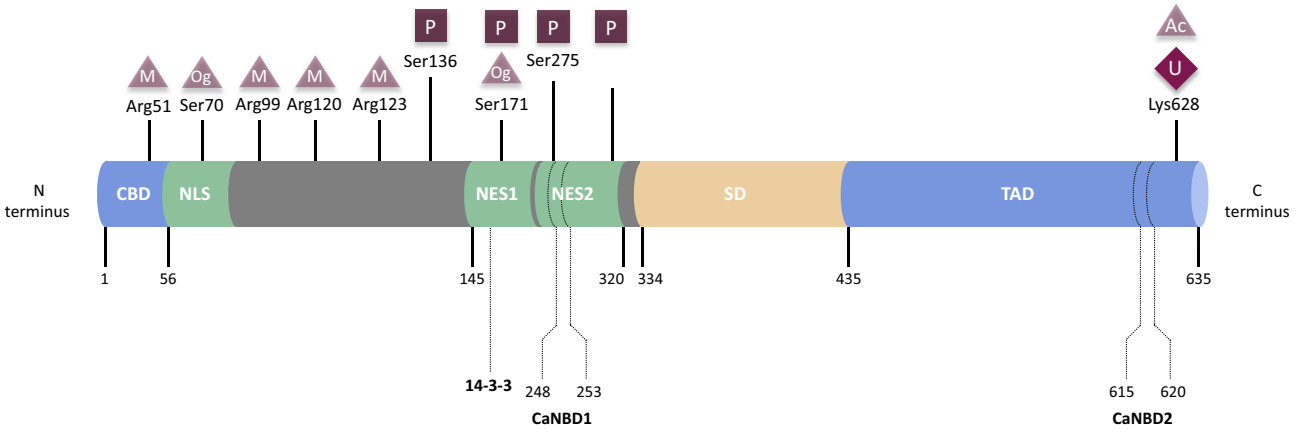
therefore be ideal targets to specifically recapitulate the physiological benefits of DR for therapeutic purposes.

Recently, work in the nematode worm *C. elegans* has highlighted a family of newly discovered cofactors, cAMP response element-binding protein (CREB)-regulated transcriptional coactivators (CRTCs), as novel modulators of aging that link energy sensing to transcriptional regulation of longevity [4,5]. Although the role of CRTCs in longevity to date has only been shown in *C. elegans*, the past 5 years have seen the discovery of novel cellular roles for CRTCs and links between CRTC dysfunction and multiple human age-related pathologies. The discovery and canonical regulation of CRTCs have been outlined extensively previously [6]. We will briefly outline new cellular roles (outputs) for CRTCs together with known CRTC regulators (inputs) before focusing on mechanistic links between CRTC regulation/function and multiple age-onset pathologies, and, finally, whether CRTCs might be targeted to promote healthy aging in mammals.

Outputs Beyond CREB: Emerging Roles for CRTCs

Initially named transducers of regulated CREB (TORCs) or mucoepidermoid carcinoma translocated protein (MECTs), CRTC family members were first identified as coactivators of the transcription factor CREB in view of their ability to induce CREB target gene expression in the absence of a cAMP stimulus [7–10]. However, recent work has identified expanding roles for CRTCs outside the confines of their name, showing that CRTCs act both as regulators of transcription factors beyond CREB and of processes other than transcription. Three isoforms of CRTC (CRTC1–3) have been described in mammals, with CRTC2 and 3 showing 32% homology with CRTC1 [8]. In humans, CRTC2 and CRTC3 are ubiquitously expressed, whereas CRTC1 is mainly expressed in the brain, with little expression in other tissues [7,11–13]. CRTC2 and CRTC3 are both expressed in the immune tissue, in B and T lymphocytes [7], as well as in lungs [13]. CRTC2 is highly expressed in liver [14] and muscle [13], whereas CRTC3 is predominantly found in white adipose tissue (WAT) and brown adipose tissue (BAT) [15]. CRTCs do not directly bind to DNA, but instead act as cofactors for bZIP transcription factors. CRTCs contain a highly conserved N-terminal coil-coil domain required for CREB binding (aa 1–56) [7], an invariant sequence matching the protein kinase A (PKA) phosphorylation consensus sequence (RKXS), a serine/glutamine rich domain, and a negatively charged C-terminus domain sufficient for transcriptional activation (last 200 aa) [8] (Figure 2). The N-terminal CREB-binding domain of CRTCs binds to the bZIP domain of CREB [16] and facilitates recruitment of the transcriptional apparatus [6–8]. However, recently the role of CRTCs as crucial transcriptional coactivators has expanded beyond CREB, and CRTCs are reported to both directly bind to and coactivate additional bZIP transcription factors, including AP-1 and activating transcription factor (ATF) 6 [17,18], and to compete with ATF3 and farnesoid X receptor (FXR) for promoter occupancy [19,20].

In addition to expanding roles in transcriptional regulation, CRTCs have now been shown to have functions beyond transcription and the nucleus. Both endoplasmic reticulum (ER)–Golgi trafficking regulation and RNA splicing have emerged as new cellular roles for CRTCs (Box 1) [21,22]. CRTC2 contains a proline-rich region at residues 334–435 which displays similarities to regions found in splicing factors (Figure 2). This domain is required for CRTC-mediated alternative splicing of transcripts with cAMP response element (CRE)-containing promoters [21]. However, CRTCs do not contain known definable RNA-binding domains, such as an RNA recognition motif, arginine/serine domain, or a K homology domain [21]. Therefore more work will be necessary to define how CRTCs modulate RNA splicing and affect spliceosome function, as well as whether CRTCs impact on known roles of RNA splicing in DR longevity [23]. In addition to splicing, CRTCs play a key role in coat protein complex II (COPII)-mediated vesicle trafficking from the ER to the Golgi via direct binding and competitive inhibition of



Structural domains of CRTC	
CBD	CREB-binding domain
NLS	Nuclear localization sequence
14-3-3	14-3-3 protein-binding domain
NES	Nuclear export sequence
CaNBD	Calcineurin-binding domain
SD	Splicing domain
TAD	Transactivation domain

Function of post-translational modifications	
■	Inhibition
▲	Activation
◆	Targeting to degradation

Type of post-translational modifications	
M	Methylation
Og	*O*-Glycosylation
P	Phosphorylation
Ac	Acetylation
U	Ubiquitination

Trends in Genetics

Figure 2. Structure and Post-Translational Modifications of CREB-Regulated Transcriptional Coactivator 2 (CRTC2) Protein. Representation of the location and function of structural domains in CRTC2 as well as the main post-translational modification sites, their type, and effect on the protein activity

SEC31. The mTORC1 pathway, a key regulator of cellular growth and organismal longevity, promotes lipogenesis via directly phosphorylating CRTC2 and de-repressing SEC31, thereby promoting cleavage and activation of the master lipogenesis regulator SREBP1 [22]. These new roles for CRTCs expand their function to new biological processes beyond transcriptional regulation of gluconeogenesis. Coupled with the effect of CRTCs on aging in *C. elegans*, CRTCs may therefore represent new cellular links between aging, metabolic homeostasis and disease risk.

Box 1. CRTCs and RNA Splicing

Through a screen for CRTC interacting partners in mammalian cells, NONO (p54nrb), a protein implicated in transcription and RNA processing, has been demonstrated to interact with CRTC2 upon cAMP signaling to regulate the tethering of the CREB/CRTC complex with RNA polymerase II on cAMP-responsive promoters [110]. Given the role of NONO in pre-mRNA splicing, it was further discovered that CRTCs affect pre-mRNA splice-site selection. These distinct effects of CRTC on alternative splicing and transcription are independent of each other, but both require functional CRE-containing promoters. The recruitment of CRTCs to the promoter by CREB is necessary to promote alternative splicing of transcripts in a cell type-specific manner. However, this does not affect the transcriptional activity of the target, suggesting mechanistically separable functions of the CRTCs. These two activities are mediated by two distinct domains on CRTC, a transactivation domain (residues 435–635) and a proline-rich domain (residues 334–434), which when mutated abrogate the effects of CRTCs on alternative splicing (see Figure 2 in main text). Although CRTCs lack any conserved canonical RNA-binding domain, they might serve as a scaffold for other proteins to form a complex to bind to and process pre-mRNA transcripts [21]. Taken together, these observations demonstrate the ability of CRTC to couple extracellular signals to gene expression through modulation of both transcriptional activity and alternative splicing.

Inputs: Post-translational Regulators of CRTCs and Aging

CRTCs are extensively post-translationally regulated, and many CRTC regulators have known roles in the modulation of aging (Table 1). CRTCs are negatively regulated through phosphorylation by AMP-activated protein kinase (AMPK) family kinases, including salt-inducible kinases 1, 2, and 3 (SIK1/2/3) [14,24,25], AMPK [10,14], and microtubule affinity regulating kinase 2 (MARK2) [26]. Phosphorylation by these kinases facilitates 14-3-3 protein binding and retention of CRTCs in the cytoplasm (Figure 3). Conversely, CRTCs are activated via dephosphorylation by the serine/threonine phosphatase calcineurin, and putatively by the suppressor of MEK null 1 and 2 (SMEK1/2), both of which modulate aging in *C. elegans* [4,27]. Dephosphorylation of CRTCs induces nuclear translocation and consequent activation of CREB targets (Figure 3) [9,28]. How CRTCs shuttle in and out of the nucleus has not been extensively studied. However, XPO1 (also known as exportin 1 or CRM1) has been shown to actively export CRTCs from the nucleus. XPO1 is a member of the importin-β superfamily of nuclear transport receptors which recognize leucine-rich nuclear export signal sequences, and its inhibition induces the nuclear sequestration of CRTCs [9]. AMPK and calcineurin have antagonistic effects on longevity in *C. elegans*, and inhibition of the sole worm CRTC family member, CRTC-1, is crucial for these effects. AMPK is a key energy sensor in all eukaryotes and a mediator of DR longevity [29]. Expression of a constitutively active AMPK increases lifespan in *C. elegans*, while also promoting phosphorylation and inhibition of CRTC-1. Conversely, inhibition of calcineurin increases lifespan in the worm and reduces CRTC-1 dephosphorylation. Blocking CRTC-1 phosphorylation at two conserved serine residues renders it constitutively nuclear and active, and suppresses both AMPK- and calcineurin-mediated longevity. Supporting a role for CRTCs inhibition in aging, inactivation of CRTC-1 via RNAi is sufficient to extend lifespan in a CREB-dependent manner [4].

In addition to the classical phosphorylation sites in CRTC2 (Ser171 and Ser275) [10,26], growing evidence points to new residues and domains that regulate the activity of this protein

Table 1. Post-Translational Modifications of CRTC2[a]

Enzyme	Type of modification	Site	Refs
Activators of CRTC2			
Calcineurin	Dephosphorylation	Ser275	[26]
SMEK1/2	Dephosphorylation	Ser171	[15]
OGT	*O*-Glycosylation	Ser70, Ser171	[33]
p300	Acetylation	Lys628	[30]
CBP	Acetylation	Lys628	[30]
PRMT6	Methylation	Arg51, Arg99, Arg120, Arg123	[34]
Inhibitors of CRTC2			
AMPK	Phosphorylation	Ser171, Ser275	[90]
SIK1	Phosphorylation	Ser307	[25]
SIK2/3	Phosphorylation	Ser171, Ser275	[24,90]
MARK2	Phosphorylation	Ser275	[26,90]
mTOR	Phosphorylation	Ser136	[22]
SIRT1	Deacetylation	Lys628	[30]
COP1	Ubiquitination	Lys628	[30,32]

[a]The table shows post-translational modifications of CRTC2 that are representative of the other CRTC isoforms. The enzymes responsible for each modification as well as the type, amino acid location, and effect (activation/inhibition) are given.

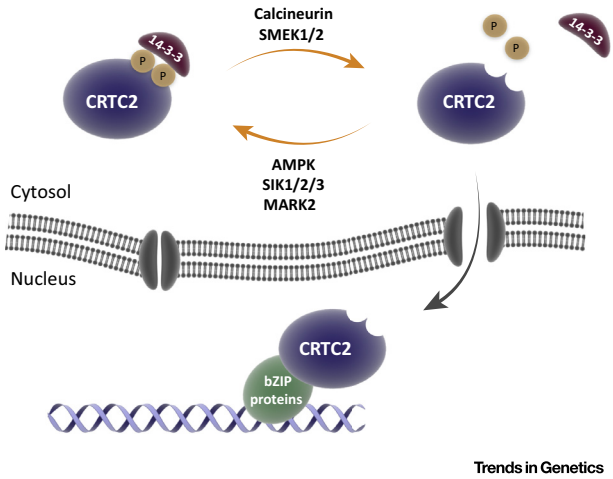


Figure 3. Regulation of CRTC2 Nuclear Localization. CRTC2 can be phosphorylated (P) by multiple LKB1-regulated kinases (AMPK, SIK1/2/3, MARK2). The phosphorylated form of CRTC2 is bound by 14-3-3 proteins and is sequestered in the cytoplasm where it is inactive. Dephosphorylation of CRTC1 by phosphatases such as calcineurin releases 14-3-3 binding and induces CRTC2 nuclear translation. In the nucleus, CRTC2 binds to bZIP transcription factors to promote transcription

and may be linked to aging (Figure 2). Gluconeogenesis during fasting is activated by glucagon stimulation of CRTC2 association with CREB-binding protein (CBP)/p300 [30]. After prolonged fasting, CRTC2 is downregulated via the nutrient-sensing deacetylase sirtuin 1 (SIRT1), which is a crucial modulator of longevity in model systems from yeast to mice [31]. In liver, SIRT1 deacetylates CRTC2 at Lys628, allowing its ubiquitination by the E3 ligase constitutive photomorphogenic protein (COP1), and targeting CRTC2 for proteasome degradation [30,32]. However, although activating SIRT1 is known to promote longevity, the role of CRTC-1 inhibition in SIRT1-mediated longevity remains untested.

In addition to phosphorylation, acetylation and ubiquitination, CRTCs are also regulated via *O*-glycosylation. *O*-Glycosyl transferase (OGT) *O*-glycosylates serines 70 and 171 in CRTC2, blocking phosphorylation and enriching nuclear CRTC2 to drive hepatic gluconeogenesis [33]. In addition, arginine methylation of CRTC2 plays an important role in the transcriptional control of hepatic glucose metabolism. Indeed, the protein arginine methyltransferase 6 (PRMT6) can promote fasting-induced transcriptional activation of the gluconeogenic program through CRTC2 [34]. As yet, little is known about how changes to these newly identified post-translational modifications impacts on CRTC function with age. However, as newly discovered inputs and outputs of CRTCs have been identified, their ties to multiple age-related pathologies have increased (Figure 4). Below we will focus on how deregulation of CRTCs inputs and outputs are causally associated with three of the greatest public health burdens in old age – namely metabolic disease, cancer, and neurodegeneration.

CRTCs and Metabolic Disorders

Homeostatic regulation of metabolism is crucial for maintaining health throughout life. Metabolic disorders such as obesity not only impact on quality of life but also reduce life expectancy and expedite the onset of multiple age-related diseases beyond type II diabetes, including cancer and neurodegeneration [35]. As such, obesity can be viewed as an accelerated-aging phenotype, adding the pandemic of obesity as a contributor to the increase in age-onset diseases [36]. CRTC family proteins are implicated in a variety of metabolic disorders in humans (Figure 4). Polymorphisms of *CRTC1* and *CRTC3* are associated with total cholesterol level in plasma, as well as with high body mass index, obesity, and hypertriglyceridemia in Mexican-Americans, Chinese populations, and psychiatric patients [15,37,38]. In addition, CRTC3 is found in the circulation, partly as a result of adipose tissue secretion in childhood obesity [39], suggesting that CRTC3 might have effects in other tissues beyond adipose and act as a

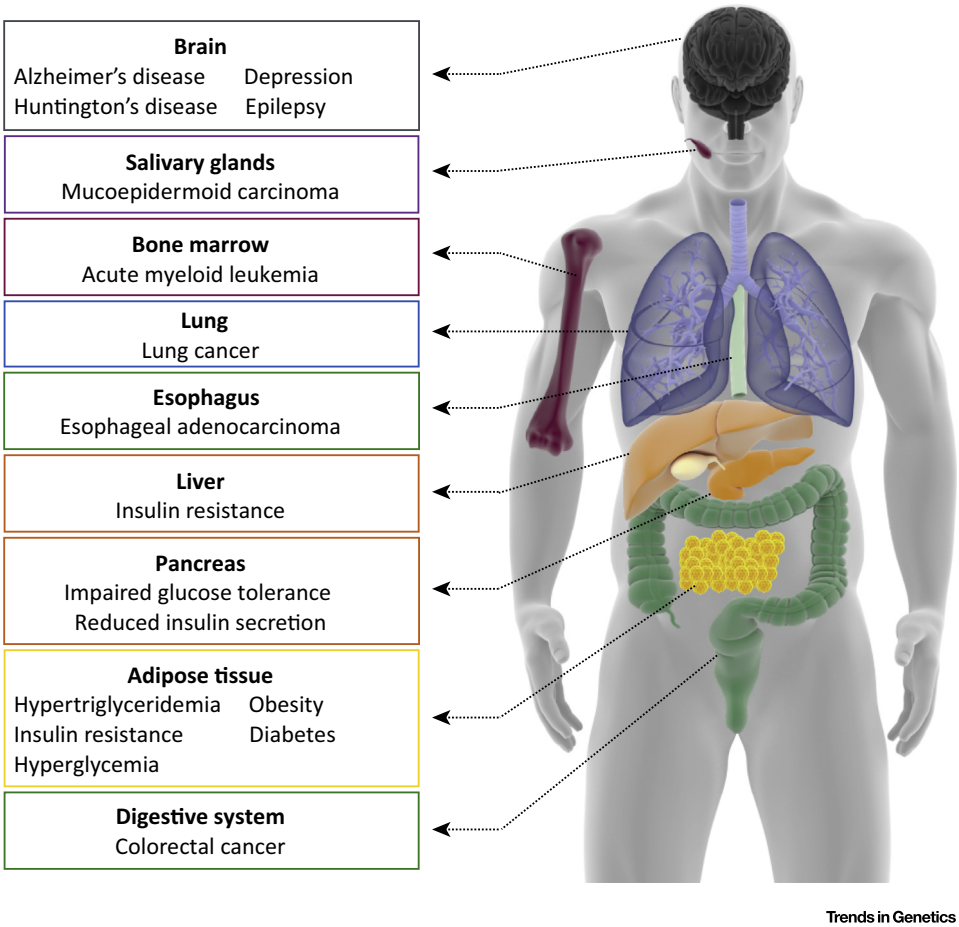


Figure 4. CRTC Dysregulation Is Linked to Disease in Multiple Organs. Human body scheme with organs in which the activity of one CRTC family member has been linked to physiological dysregulation or disease

'physiological messenger' during obesity. Most recently, polymorphisms in the *CRTC1* locus show a significance genome-wide association with body fat percentage. This association is sex-specific, being stronger in women compared to men [40]. Furthermore, in the same study, two variants present in the intron 1 of *CRTC1* showed histone marks characteristic of active transcription and RNA polymerase II binding [40]. Epigenetic deregulation of *CRTC* genes may therefore contribute to the development of metabolic disorders, and this is a key area to be explored in the future.

Lipid Metabolism

In murine models, CRTC family members have also been causally linked to obesity and metabolic disease. Homozygous mice mutant in *Crtc1* develop obesity, while CRTC2 contributes to insulin resistance and lipogenesis, and deregulation of both CRTC1 and 2 enhances gluconeogenesis and leads to high blood glucose. Lastly, CRTC3 promotes obesity through disruption of catecholamine signaling [11,15,22,41]. CRTC3 is highly expressed in WAT and less in BAT [15]. CRTC3 mutant mice have smaller adipocytes in WAT and 50% lower adipose mass compared to wild-type animals. Under HFD (high-fat diet) conditions, *CRTC3* mutants are protected from the development of obesity, insulin resistance, and hepatic steatosis. This suggests that CRTC3 may promote obesity by attenuating catecholamine signaling. One

potential mechanism for this may be upregulation of the regulator of G protein signaling (RGS2), which in turns disrupts lipolysis and fatty acid oxidation [15].

Recently, CRTC2 has also been implicated in lipid metabolism via a novel non-transcriptional function. CRTC2 controls hepatic lipid metabolism by regulation of sterol regulatory element-binding protein 1 (SREBP1). SREBP1 is a transcription factor synthesized as an inactive precursor bound to the ER. In response to insulin signaling, SREBP1 is transported to the Golgi through COPII-mediated vesicle trafficking, and then shuttled to the nucleus to induce the expression of genes involved in cholesterol and fatty acid synthesis [42]. CRTC2 modulates COPII-dependent SREBP1 activation via an inhibitory association with SEC31, a subunit of the COPII complex, at Trp143. Phosphorylation of CRTC2 by mTOR dissociates the CRTC2/SEC31 interaction and releases SEC31, promoting its binding to another COPII complex subunit, SEC23. This process allows increased SREBP1 activation and promotes lipogenesis [22]. Overall, these data expand metabolic regulation by CRTC family proteins beyond their well-defined role in gluconeogenesis to lipid metabolism, and suggest that such CRTC deregulation might be a central link in the loss of metabolic homeostasis with age.

Autoimmune Diseases

Obesity promotes insulin resistance in part by activating the innate immune system. In this regard, obesity causes an M2-to-M1 (alternatively activated M2 macrophages to classically activated M1 macrophages) shift in adipose tissue that leads to insulin resistance [43]. CRTC3 controls the interconversion from M1 to regulatory macrophages (M2b) [44], and alternatively activated M2 macrophages can promote insulin sensitivity [45]. Increases in cAMP signaling promote M2 macrophage polarization, and CRTC2 and CRTC3 are required for this induction [46]. This induction does not seem to take place directly via CRTC/CREB regulation of M2 marker genes because these genes do not have conserved CREB binding sites on their promoters [46]. In contrast to the effects of CRTCs on HFD-induced obesity described above, these data show that CRTC2/3 promote M2 polarization in macrophages to protect adipose tissue from insulin resistance during obesity. However, the positive or negative role of CRTCs in immune response remains unclear because CRTC2 also promotes autoimmune disease by stimulating type 17 T helper (Th17) cell differentiation [47]. Th17 cells promote the clearance of pathogens, but they are also implicated in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, and CRTC2 promotes Th17 differentiation from CD4 T cells, stimulating the expression of cytokines IL-17A and IL-17F, and increasing the immune response [47]. More work is therefore necessary to elucidate the roles of CRTCs in autoimmune diseases and inflammation, in addition to how CRTC function in macrophages is linked to other disorders such as cancer (see cancer section).

Glucose Homeostasis

Acute regulation of metabolic flexibility is particularly crucial during fasting, and short-term or intermittent fasting has been shown in multiple species to restore metabolic homeostasis and positively impact lifespan [48]. CRTCs were also identified as key regulators of the metabolic shifts during fasting [14]. In response to fasting, SIK proteins (SIK1-3) are phosphorylated, reducing CRTC2 and CRTC3 phosphorylation and triggering gluconeogenic gene expression [14,24,25,32,49,50]. Nuclear translocation of CRTC2 enhances the transcription of CREB-dependent gluconeogenic genes including phosphoenolpyruvate carboxykinase (*PEPCK*) and glucose-6-phosphatase (*G6P*) [14,32]. *PEPCK* and *G6P* are also targets of the glucocorticoid receptor (GR), and CRTC2 functions as a coactivator that enhances the transcriptional activity of GR [51]. Unlike control animals, animals with constitutively active CRTC2 in liver are unable to reduce blood glucose concentrations following insulin administration [52]. In support, *Sik2* knockout mice have increased blood glucose and serum insulin levels, as well as an increased population of larger fat cells, phenotypes that all depend upon CRTC2 [53]. In these mutant

animals, the CRTC2/CREB complex leads to increased expression of *Atf3* and subsequent downregulation of the glucose transporter GLUT4 and adiponectin in WAT, affecting whole-body glucose metabolism [53]. Thus, constitutive activation of the CRTC2 pathway in liver is sufficient to promote insulin resistance. Conversely, *Crtc2* knockout mice show reduced gluconeogenic gene expression (G6Pase and PEPCK), lowered hepatic glucose production during fasting, and reduced circulating glucose, insulin, triglycerides, and cholesterol concentrations [41,52]. In addition to transcriptional regulation of gluconeogenic gene expression, CRTCs also modulate gene expression by epigenetic changes: CRTC2 modulates gluconeogenic gene expression during fasting and diabetes by promoting histone H3 acetylation at Lys9 (H3K9Ac) [54]. Recruitment of the lysine acetyltransferase 2B (KAT2B) and WD repeat-containing protein 5 (WDR5) by CRTC2 is required for this acetylation [54].

In addition to its effects on hepatic glucose homeostasis, CRTC2 has been implicated in the regulation of pancreatic metabolism [26,55–57]. CRTC2 has a protective role in pancreas, promoting insulin secretion and cell survival [56]. CRTC2 contributes to β cell proliferation by inducing the expression of the antiapoptotic *Bcl2* gene [58]. In addition, CRTC2 promotes insulin secretion by stimulating MAF bZIP transcription factor A (MAFA) expression, which is a β cell-restricted factor [55]. *In vivo*, mice with β cell-specific knockout of CRTC2 secrete less insulin in response to oral glucose gavage, suggesting that CRTC2 is required for β cell function [55].

Although multiple groups have shown that activated CRTC proteins translocate to the nucleus to regulate glucose metabolism, Han *et al.* showed that this shift is not always required [34]. The protein arginine methyltransferase PRMT6 binds to CRTC2 and enhances CRTC2-dependent CRE-containing target gene expression to induce the gluconeogenic program in the liver. However, methylation of conserved CRTC2 arginine residues by PRMT6 does not affect its subcellular localization [34]. These results suggest that CRTC proteins can impact on metabolic signaling from different subcellular compartments, and that their functions are not restricted to nucleus. Clarifying the importance of CRTCs subcellular localization and their conserved domains in metabolism should therefore reveal how changes to CRTCs function with age impact on metabolic disease risk.

Neuronal Regulation of Metabolism

In *Drosophila*, CRTC family proteins link metabolism to stress response. Expression of the *Drosophila crtc* is increased in response to fasting [59]. *Crtc* mutant flies are sensitive to fasting and oxidative stress (paraquat), and have lower stored glycogen and lipids. Expression of *Crtc* in neurons rescues the starvation- and paraquat-sensitive phenotypes, as well as lipid levels, indicating that Crtc regulates the stress response and lipid storage cell-nonautonomously from the nervous system [59]. Furthermore, overexpression of *Crtc* in sNPF (short neuropeptide F) neurons of wild-type flies further enhances starvation resistance [60]. Crtc maintains metabolic homeostasis in response to stress and inflammation from neurons to gut, regulating the expression of short neuropeptide F (sNPF), an ortholog of mammalian neuropeptide Y. Crtc together with CREB binds to the sNPF promoter to regulate its expression, and the sNPF receptor in the gut mediates the effects of the neuronal Crtc/sNPF pathway [60]. In support of a key role in central regulation of metabolism for this pathway, salt-inducible kinase (SIK) in flies regulates body lipids, glycogen, and fasting resistance from neurons through Crtc [61].

In addition to peripheral regulation, for example by liver, adipose, muscle, and pancreas, the central nervous system crucially maintains systemic metabolic homeostasis during aging [62]. Supporting the data from *Drosophila,* CRTCs in the brain can also impact on organismal metabolism in mammals. By immunolocalization, mammalian CRTC2 is detected both in the

nuclei and cytosol of brain tissues. CRTC2 nuclear expression is present in the hippocampus as well as in the trigeminal and hypoglossal nuclei [63], and its cytoplasmic expression has been detected specifically in the lateral hypothalamic area of dissected tissues [63] and also in hypothalamic cell cultures [63,64]. Therefore CRTC2 likely has cell type-specific functions in brain that depend upon its subcellular localization. Hypothalamic CRTC2 phosphorylation reflects nutrient status, being dephosphorylated in fed mice, phosphorylated after fasting, and dephosphorylated after glucose [63]. Coupled with this, CRTC2 translocates into hypothalamic cell nuclei during exposure to high glucose [64]. *CREB* expression is also increased after glucose treatment [64]. In addition, the expression of CREB target genes, such those encoding insulin receptor substrate 2 (IRS2) and thyrotropin releasing hormone (TRH), requires activation of CRTC2 via inhibition of AMPK in hypothalamic cells [63,64]. These data demonstrate that the AMPK/CRTC2/CREB axis is conserved in the mammalian central nervous system.

Work in *C. elegans* suggests that regulation of the neuronal AMPK/CRTC/CREB axis underlies the effects of CRTCs on systemic longevity and metabolism. Lifespan extension via ubiquitous activation of AMPK is associated with remodeling of mitochondrial metabolism. Blocking phosphorylation of CRTC-1 by AMPK only in neurons is sufficient both to suppress AMPK longevity and reverse the effects on metabolism. Furthermore, neuronal CRTC-1 activation alone can drive fragmentation of mitochondrial networks in muscle. Neuronal CRTC-1 modulates systemic longevity and metabolism via octopamine, a monoamine neurotransmitter, and mutations in octopamine synthesis enzymes derepress the effect of neuronal CRTC-1 on AMPK- and calcineurin-mediated longevity [5]. These data establish a CRTC-1 cell-nonautonomous role in communicating perception of energy status in neurons to systemic regulation of metabolism and lifespan in *C. elegans*, and examining whether these effects are conserved in mammals now becomes a priority [4,5]. Initial data suggest that the effect of neural regulation of metabolism by CRTCs on longevity may be conserved in mammals [65]. Mice lacking the transient receptor potential cation channel subfamily V member 1 (TRPV1), a pain receptor, are long-lived putatively because of inactivation of the CRTC1/CREB pathway in sensory neurons [65]. In TRPV1 neurons, CRTC1 induces the release of neuropeptide calcitonin gene-related peptide (CGRP), which in turn antagonizes insulin release from pancreatic β cells [65]. Given that CRTC2 regulates pancreatic β cell function by induction of the β cell-restricted transcription factor, MAFA, promoting insulin gene expression and secretion [55], it would be interesting to determine if CRTC1 and CRTC2 might indirectly interact from different tissues to regulate systemic metabolism.

Taken together, although CRTCs were first discovered because of their role in modulating CREB-mediated gluconeogenesis in fasting, as new metabolic roles for CRTCs are discovered CRTC family proteins are emerging as key links between organismal metabolism and aging. CRTCs may therefore provide novel therapeutic avenues for maintaining metabolic homeostasis in old age.

CRTCs and Cancer Cell Proliferation

Another crucial disease for which age is a potent risk factor is cancer (Figure 4), and CRTCs have been implicated in carcinogenesis in several conditions, including lung cancer, colorectal cancer, acute myeloid leukemia, mucoepidermoid carcinoma, and esophageal adenocarcinoma [4,5,66–74]. Mechanistic links between CRTCs and cancer are only beginning to emerge but often involve hypophosphorylation of CRTC1 and upregulation of target gene expression. Liver kinase B1 (LKB1) is a serine/threonine kinase that acts as a crucial direct activator of AMPK and the 12 AMPK-related kinases [75]. LKB1 is downregulated in a subset of human esophageal cancer cell lines and in samples from patients with lung cancer or cervical carcinoma [67]. Because multiple AMPK family kinases (i.e., SIK2) can directly inhibit CRTCs,

LKB1 loss therefore indirectly leads to CRTC1 hypophosphorylation and activation, and to unregulated expression of oncogenic CRTC1 targets such as glycosylphosphatidylinositol (GPI)-anchored metastasis-associated protein (*LYPD3*), cyclooxygenase-2 (*COX2*), and the long non-coding RNA LINC00473 [66,68,69,76]. Dysregulation of LKB1/SIK2/CRTC1 pathway may therefore contribute to cancer progression.

Mucoepidermoid carcinoma (MEC) is the most common malignant form of salivary gland tumor. MECs are induced by recurrent chromosomal translocations that fuse the segment encoding the N-terminal CREB-binding domain (CBD) of CRTC1 with substantial portions of the coding region of another transcriptional coactivator, mastermind-like (MAML)2 [77]. This specific chromosomal translocation joins exon 1 of *CRTC1* to exons 2–5 of *MAML2*, resulting in the expression of a *CRTC1–MAML2* fusion. This CRTC1–MAML2 fusion protein controls the expression of genes that contribute to tumor growth [7]. One such target is an autocrine signal governed by amphiregulin (AREG)/epidermal growth factor receptor (EGFR) [70]. Secreted AREG ligand then activates EGFR signaling in an autocrine manner to induce cell growth and survival of MEC cells [70]. An additional molecular mechanism linking CRTC1–MAML2 to tumorigenesis is via aberrant activation of CREB. CRTC1–MAML2 fusion is constitutively nuclear, and binds to CBP/p300, recruiting CREB, and enhancing the oncogenic activity of CREB [72]. In addition, >800 human genes have been identified as CRTC1–MAML2 targets, and these are involved in cellular movement, development, death and survival, growth and proliferation, cell-to-cell signaling and interaction, and metabolism [70,71], indicating that CRTC1–MAML2 might be a promising therapeutic target.

Taken together, these data establish CRTC proteins as potential targets for the treatment of cancer [78]. Indeed, this idea is supported by data on endogenous regulation of CRTC1 by microRNA-22. miR-22 has been suggested to have an antitumor role in acute myeloid leukemia (AML) [79]. During leukemia, miR-22 is downregulated via transcriptional regulators TET1/GFI1/EZH2/SIN3A, which increase histone H3K27 trimethylation (me3) at the miR-22 promoter. Repression of miR-22 derepresses CRTC1, and upregulates oncogenic CREB targets (*CDK6*, *HOXA7*, *BMI1*, *FASN*, and *HMGA1*) [79]. However, the role of CRTCs as a friend or foe of cancer remains controversial. In addition to putative oncogenic roles of CRTCs during cancer development, recent data also suggest that *CRTC2* can act as a lymphoma tumor-suppressor gene. CRTC2 plays an important role in maintaining genome integrity by promoting transcription of DNA mismatch repair (MMR) genes *EXO1*, *MSH6*, *PMS1*, and *POLD2*. *CRTC2* knockout HeLa cells have reduced MMR activity, which in turn leads to a 25-fold higher spontaneous mutation rate [80]. Furthermore, *CRTC2* expression is reduced in lymphoma cell lines and patient samples. This reduction results from low levels of acetylated histone H3 at the *CRTC2* promoter, leading to increased mutation frequency as well as microsatellite instability [80]. These data suggest that deregulation of CRTCs and their targets may have causal links to multiple forms of cancer, but the directionality of these links may well be context-specific. Therefore if and how CRTCs might be targeted for cancer therapeutics will depend upon the specific malignancy in question.

CRTCs Function in Learning, Memory, and Neurodegenerative Diseases

CREB has an evolutionarily conserved fundamental role in regulating memory formation and memory consolidation [81–83]. The cAMP–CREB pathway controls memory for example by enhancing synaptic transmission or increasing neuronal excitability [81]. Modulating CREB levels or its binding partner the histone acetyltransferase CBP (CREB binding protein) by overexpression or knockout results in either enhanced memory formation or memory deficits, respectively, in mammalian models [81]. Even though CREB phosphorylation at Ser133 is an important activating post-translational modification, it is not always sufficient to induce CREB-

dependent gene transcription [84], suggesting that additional mechanisms regulate CREB function. CRTC1 has therefore been mainly studied in the context of memory formation and has been characterized for its role in neurodegenerative diseases [85]. Intriguingly, in brain tissue, activation of CRTC1 seems to be beneficial, in contrast to the detrimental effects of CRTC hyperactivation discussed earlier in metabolic disease and cancer.

Crtc1 knockout mice do not display any brain developmental defects [11]; however, these mouse models have been informative in highlighting the importance of CRTC1 in neuronal function [86,87]. *Crtc1* knockout mice display a treatment-resistant depression-like phenotype [87] as well as symptoms associated with mood disorders such as impulsive aggressiveness, social withdrawal, and decreased sexual motivation. These phenotypes are likely caused by decreased levels of dopaminergic and serotoninergic activity in the mouse prefrontal cortex, and by reduced expression of CREB susceptibility genes involved in neuroplasticity [86].

Synaptic activity plays a major role in regulating CRTC1 function in neurons. Synaptic stimuli induced by AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) and NMDA (*N*-methyl-D-aspartic acid)-type glutamate receptors, LVGCC (L-type voltage-gated calcium channels) calcium influx, or GABA (γ-aminobutyric acid) receptor activation are necessary to trigger CRTC1 nuclear import and transcription-dependent plasticity effects. Indeed, CRTC1 localizes to dendrites and spines in electrically silenced hippocampal neurons and translocates to the nucleus in a calcium- and calcineurin-dependent manner upon stimulation [88]. Post-translational modifications and subsequent changes in CRTC1 subcellular localization integrate this signal. Activation of synaptic NMDA or AMPA glutamate receptors, or of LVGCC, induces a local increase in calcium concentration at the synapse (as opposed to a general rise in intracellular calcium), which triggers the dynein-mediated retrograde transport of CRTC1 along microtubules from the synapse to the nucleus [89]. The activity-dependent nuclear shuttling of CRTC1 in neurons occurs within minutes and, conversely, once the stimulus stops, CRTC1 returns to the cytoplasm in 30 minutes [90]. In hippocampal neurons, basal constitutive phosphorylation of CRTC1 at Ser151 and Ser245 is controlled by SIK2/AMPK and MARK2 enzymes, whereas stimulus-triggered dephosphorylation is mediated by the phosphatase calcineurin [90]. Dephosphorylation of three serine residues (Ser64, Ser151, Ser145) on CRTC1 is necessary and sufficient for dissociation from 14-3-3 protein and nuclear accumulation [89]. Activity-stimulated dephosphorylation of CRTC1 at Ser151 can induce transcriptional activation of CRTC1 in mouse hippocampus [91]. Nuclear CRTC1 then drives CRE-dependent transcription even in the absence of CREB phosphorylation on Ser133 [90]. However, CREB is required for CRTC1 to bind to CRE sites in the promoters of immediate early genes (IEG) in an activity-dependent manner.

Long-term potentiation (LTP) is an activity-dependent long lasting increase in synaptic strength. LTP is a form of synaptic plasticity which requires gene transcription and protein synthesis, and is considered to be an electrophysiological model for the basic mechanisms involved in learning and memory formation [92,93]. CRTC1 in neurons senses the coincidence of calcium and cAMP signals, and induces a CREB-dependent transcriptional response leading to enhanced synaptic transmission (through, for example, the expression of brain-derived neurotrophic factor, BDNF) [12,94,95]. CRTC1 is involved in the maintenance of late-phase LTP in mouse hippocampal slices [12], and induction of late-phase LTP triggers robust nuclear accumulation of CRTC1 in neurons. Consistently, nuclear localization of CRTC1 facilitates late-phase LTP induction in mouse hippocampal slices, whereas expression of a dominant negative CRTC1 suppresses the maintenance of late-phase LTP [94,96]. Similarly, increasing CRTC1 or CREB function in excitatory dentate granule cells of the dentate gyrus region of the dorsal hippocampus of mice is sufficient to enhance memory stabilization as well as memory reactivation without compromising memory quality in a fear conditioning assay, suggesting a region-specific role for

CRTC1 in controlling memory [97]. More recent work has shown that, in mouse hippocampus, CRTC1 induces chromatin changes following associative learning to promote sustained target gene transcription and enhance memory strength [98].

In *Drosophila*, Crtc nuclear activity is required specifically in the mushroom body of the fly for memory formation induced in appetitive long-term memory (fLTM: a single training preceded by a period of fasting). The fasting period downregulates the insulin signaling pathway in *Drosophila*, which normally phosphorylates Crtc via SIK2 leading to Crtc degradation [59]. Nuclear translocation of Crtc after fasting can be mimicked by the expression of a constitutively active form of Crtc, which is necessary and sufficient to form fLTM in the mushroom body. Supporting this mechanism, *Drosophila chico* mutants that display reduced insulin signaling mimic fLTM through the activation of Crtc in the mushroom body [99]. Interestingly, the *Drosophila chico* mutation, which extends lifespan via downregulation of the insulin signaling pathway [100], has been shown to activate Crtc to promote beneficial effects [59,99]. These observations contrast with the requirement for CRTC-1 inhibition in AMPK-mediated longevity in *C. elegans* [4], suggesting that CRTCs might display opposing roles in the regulation of lifespan or metabolism, and in neuronal function and memory. Lastly, in addition to being involved in the formation of memory in *Drosophila*, the CRTC/CREB complex is also involved in early maintenance of long-term memory (LTM), targeting genes required for memory maintenance and extinction in mushroom bodies [101].

Alzheimer's Disease (AD)

Data in mammals and *Drosophila* therefore highlight the importance of CRTC in neuronal plasticity and memory formation. Not surprisingly, therefore, CRTC deregulation has been linked to the pathogenesis of age-onset diseases associated with memory defects such as AD and Huntington's disease. In the APPsw/ind mouse model of AD (transgenic mice expressing the mutant human APP695 isoform harboring the familial AD-linked Swedish K670N/M671L and Indiana V717F mutations in neurons), accumulation of the toxic amyloid β peptide in neurons reduces calcineurin activity by disrupting calcium influx through L-type calcium channels. Calcineurin-dependent CRTC1 dephosphorylation and activation is thus impaired, leading to disruption of the CRTC1-dependent gene expression program. On a functional level, the APPsw/ind mice display a hippocampus-dependent memory deficit in the Morris water maze spatial memory task, and this correlates with a specific reduction in the expression of CRTC1-dependent genes normally upregulated during training [102]. Furthermore, overexpression of CRTC1 by AAV (adeno-associated virus) delivery in mouse hippocampus improves learning and memory of the AD mice in the Morris water maze test, without affecting the performance of wild-type animals [91]. Human data support the hypothesis that CRTC1 function is altered in AD patients, and might therefore represent a therapeutic target. Studies reveal a reduction of both total and phosphorylated CRTC1, as well as decreased expression of CRTC1 targets, in human hippocampal samples of AD pathological cases (classified according to Braak stages) [91,103]. In addition, DNA methylation at the *CRTC1* promoter is significantly lower in hippocampus samples of AD patients, and methylation levels inversely correlate with phospho-tau and β-amyloid pathology, suggesting a link between CRTC1 activity and neurodegenerative pathophysiology.

Huntington's Disease (HD)

HD is caused by an abnormal autosomal dominant glutamine repeat expansion of 3 nucleotides (CAG) in the huntingtin (HTT) protein, leading to cognitive, psychiatric, motor dysfunction, and loss of autonomy during the final stages of the disease (Huntington's Disease Society of America; www.hdsa.org). Similarly to Alzheimer's Disease, CRTC1 expression levels are decreased *in vivo* in postmortem striatal tissue from HD patients, in the striatum of various transgenic mice models (NLS-N171-82Q, R6/2 and HdhQ111 HD), and *in vitro* in STHdhQ111

cells expressing mutant HTT [104]. Mutant HTT interacts with SIRT1 and disrupts SIRT1-mediated activation of CRTC1 [105]. Under normal conditions, SIRT1 promotes deacetylation of CRTC1 (lysines 13 and 20) and activates CRTC1 by promoting its dephosphorylation and interaction with CREB, which leads to activation of neuronal target genes such as *Bdnf*. Interestingly, this activating effect of SIRT1 on CRTC1 in neurons contrasts with the effect of SIRT1 on CRTC2 in the liver. Indeed, fasting induces SIRT1-mediated deacetylation of CRTC2 and its subsequent ubiquitination and degradation by the proteasome [29,31]. Such opposing regulation by SIRT1 of different CRTC isoforms might indicate tissue-specific modulation and physiological roles for the CRTCs. In a mouse model of HD (R6/2), SIRT1 overexpression is neuroprotective, and CRTC1 and CREB are at least partially required for SIRT1-mediated neuroprotection in primary cortical neurons. The requirement for CRTC1 in SIRT1-mediated neuroprotection *in vivo* in a HD mouse model remains to be explored [105].

CRTC plays an important role in the expression of an additional key metabolic regulator, the transcriptional coregulator PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) [12]. PGC-1α is a master regulator of mitochondrial biogenesis, energy homeostasis, adaptive thermogenesis, and glucose metabolism whose expression is impaired in HD [106,107]. Chaturvedi *et al.* therefore asked whether PGC-1α might mediate the beneficial effects of CRTC1 in HD models. CRTC1 overexpression in wild-type or HTT-expressing striatal cells (Htt cells) increased PGC-1α promoter activity, the expression of downstream targets (NRF-1, Tfam, CytC), as well as mitochondrial DNA content, mitochondrial activity, and mitochondrial membrane potential [104]. Consistent with this result, CRTC1 knockdown enhances mitochondrial dysfunction caused by a mitochondrial toxin (3-NP, inhibits complex II of the electron transport chain) in Htt cells, and this effect can be partially rescued by PGC-1α overexpression. Finally, specific knockdown of CRTC1 in the striatum in NLS-N171-82Q HD transgenic mice induces neurodegeneration and cell death [104].

Given the results of these studies, CRTCs represent a potential therapeutic target for various age-related diseases ranging from metabolic dysfunction to neurological disorders. However, recent work highlights the complex and sometimes opposing roles of CRTCs in aging versus specific diseases. It is therefore necessary to better elucidate the mechanism of CRTCs activity, and how their dysregulation affects physiology and pathogenesis in aging individuals.

Concluding Remarks

As recent work has conclusively shown, CREB-regulated transcriptional coactivators play broader cellular roles than was initially thought (Boxes 2 and 3). Being neither regulated by CREB nor exclusively transcriptional coactivators, perhaps the time has come for a rethink of their – with hindsight – somewhat myopic name. Although 'cAMP-regulated transcriptional coactivator' has been suggested, until more work is done on these understudied proteins a name that adequately encompasses their multiple roles and binding partners

Outstanding Questions

How do CRTCs become deregulated with age?

Which cellular role of CRTCs contributes to their effect on organismal aging?

Is the role of CRTCs in AMPK longevity conserved?

Can we target CRTCs for neuroprotective benefits?

Do CRTCs have antagonistic effects on aging and disease in different tissues?

Can CRTC family members cell-non-autonomously coregulate each other across tissues to regulate metabolism and aging?

Box 2. CRTCs and Stem Cell Regulation

Crtc has recently been described as an inducer of intestinal stem cell proliferation (Figure 5). In *Drosophila*, dietary L-glutamate acts on the metabotropic receptor mGluR to influence cytoplasmic Ca^{2+} oscillations via phospholipase C and IP3Rc, leading to an overall increase in cytoplasmic calcium. High cytoplasmic calcium induces nuclear translocation of Crtc through the activation of its upstream phosphatase calcineurin [108]. CRTC therefore integrates signals from the diet to induce intestinal stem cell activity in *Drosophila*. These discoveries are the first steps in the study of stem cell regulation by CRTCs, and suggest that CRTCs may have a role in other types of stem cells. For instance, CRTC1/2/3 have been identified in human testis samples [109], suggesting a role in the development of germ stem cells.

Key Figure

Physiological and Cellular Processes Modulated by CRTCs

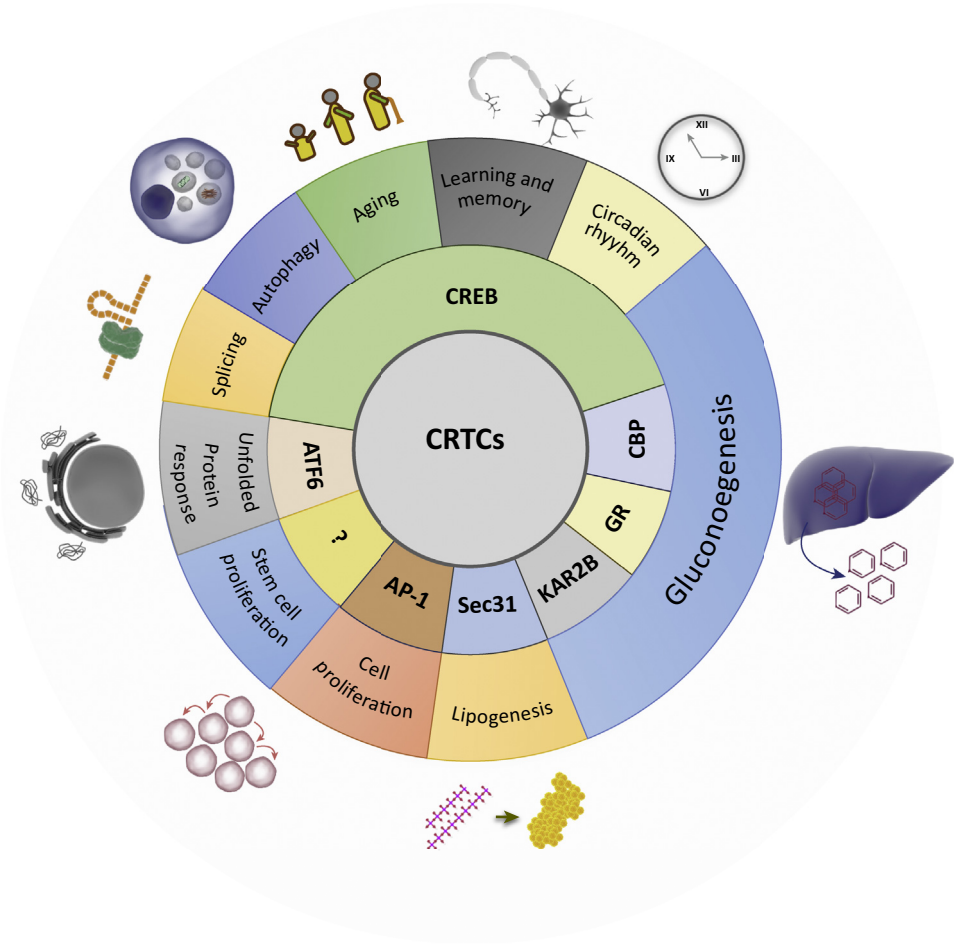


Figure 5. The inner circle lists the proteins known to interact directly with at least one CRTC family member. The outer circle represents the physiological and cellular processes specifically controlled by the interaction between CRTC and its interacting partner. CRTC can either interact with a unique partner to modulate a unique pathway (e.g., CRTC–SEC31, influencing lipogenesis), or multiple pathways (e.g., CRTC–CREB, acting on splicing, autophagy, etc.), or can interact with multiple proteins to act on the same physiological process (e.g., CRTC–CREB or CRTC–CBP, modulating gluconeogenesis)

(Figure 5, Key Figure) may be beyond reach. As discussed above, despite sequence similarity, different CRTC family members have differing functions and roles in both physiology and disease (Table 2). This is in part due to their largely non-overlapping tissue expression patterns, but may also reflect how recent the many newly described roles for these proteins are – full characterization of all three proteins is still lacking. The role of CRTCs in organismal aging itself has only been described in *C. elegans*, but, as outlined here, deregulation of mammalian CRTCs is linked to multiple age-onset pathologies. CRTCs can be both protective and destructive depending on the disease in question. Therefore more work will be necessary to determine how different CRTCs become deregulated with

Table 2. Expression and Physiological Roles of CRTC Isoforms[a]

Organism	Isoform	Tissue-specific expression	Physiological process regulated	Refs
 Mouse	CRTC1	Brain	Energy balance, fertility	[11]
			(Hippocampus) memory formation	[98]
			(Suprachiasmatic nuclei) circadian rhythm	[118,119]
	CRTC2	White adipose tissue	Insulin resistance	[53]
		Lung	Unknown	[13]
		Liver	Regulation of lipogenesis	[22]
			Gluconeogenesis	[14,30,32]
		Muscle	Mitochondrial biogenesis	[13]
		Immune system	Macrophage M1 to M2 interconversion	[46]
			(Promotes insulin sensitivity)	
			Th17 cell differentiation	[47]
		Brain	Hypothalamic glucose sensing	[63,64]
		Pancreas (β cells)	Insulin secretion	[26,55,56]
	CRTC3	Lung	Unknown	[13]
		White adipose tissue	Attenuates response to catecholamine signals	[15]
		Brown adipose tissue	Unknown	[15]
		Immune system	Macrophage M1 to M2 interconversion	[72]
 *Drosophila*	Crtc	Neurons	Central regulation of metabolism	[59–61]
			(Mushroom body) memory formation	[99,101]
			Circadian rhythm	[120]
		Intestine	Stem cell proliferation	[108]
 *C. elegans*	crtc-1	Neurons	Lifespan	[4,5]
			Central regulation of metabolism	
		Intestine	Unknown	[4]

[a]The table lists CRTC isoforms in mouse, *Drosophila*, and *Caenorhabditis elegans*, their tissue-specific expression, and physiological roles.

age across different tissues (see Outstanding Questions). However, what is increasingly clear is that age-onset deregulation of CRTC family members underlies the pathophysiology of age-onset disorders beyond high blood glucose and type II diabetes. CRTCs are therefore molecular examples of geroscience targets – linking age to a multitude of diseases of the elderly.

Box 3. CRTCs in the Circadian Rhythm

The circadian rhythm is a tightly regulated internal biological clock which modulates various physiological processes and undergoes changes with age [111–114]. In particular, dysregulation of circadian rhythm is associated with alterations in metabolism and development of cancers [115–117]. The master circadian pacemaker is located in the suprachiasmatic nuclei (SCN) in the hypothalamus, and responds to light through retinal photoreceptors. CRTC1 displays both rhythmic expression pattern in the SCN and strong nuclear accumulation following light stimulation [118]. Indeed, upon light exposure, intraneuronal calcium levels rise, leading to both phosphorylation of CREB and nuclear import and activation of CRTC1. The resulting CRTC–CREB complex induces the expression of the major circadian rhythm regulator, *Per*, as well as the kinase *Sik1*. SIK1 serves as a negative feedback mechanism by phosphorylating CRTC1 and deactivating it to suppress further effects of light on the clock [119]. In addition to modulating circadian clock in response to light, a more recent study in *Drosophila* suggests a light-independent role of Crtc in sustaining circadian behaviors by acting as a transcriptional coactivator of another essential circadian gene, *timeless* [120]. These findings thus highlight a novel role for CRTCs in regulating another major physiological process that becomes deregulated with age, the circadian clock (see Figure 5 in main text).

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