**Serveur Académique Lausannois SERVAL serval.unil.ch**

# Author Manuscript

## Faculty of Biology and Medicine Publication

**This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.**

Published in final edited form as:

**Title:** Emerging Roles of CREB-Regulated Transcription Coactivators in Brain Physiology and Pathology.

**Authors:** Saura CA, Cardinaux JR

**Journal:** Trends in neurosciences

**Year:** 2017 Dec

**Issue:** 40

**Volume:** 12

**Pages:** 720-733

**DOI:** 10.1016/j.tins.2017.10.002

**In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.**

serval
serveur académique lausannois

UNIL | Université de Lausanne
Faculty of Biology and Medicine

# Emerging Roles of CRTCs in Brain Physiology and Pathology

Carlos A. Saura[1,*] and Jean-René Cardinaux[2,*]

[1]Institut de Neurociències, Department de Bioquímica i Biologia Molecular, Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Universitat Autònoma de Barcelona, Bellaterra, Spain. [2]Center for Psychiatric Neuroscience and Service of Child and Adolescent Psychiatry, Department of Psychiatry, University Medical Center, University of Lausanne, Switzerland

*Correspondence:
carlos.saura@uab.es (C.A. Saura) and
Jean-Rene.Cardinaux@chuv.ch (J-R. Cardinaux)

Keywords: CREB, memory, synaptic plasticity, neurodegeneration, major depression.

## Abstract

The brain has the ability to sense, coordinate and respond to environmental changes through biological processes involving activity-dependent gene expression. CREB-regulated transcription coactivators (CRTCs) have recently emerged as novel transcriptional regulators of essential biological functions, while their deregulation is linked to age-related human diseases. In the brain, CRTCs are unique signaling factors that act as sensors and integrators of hormonal, metabolic, and neural signals contributing to brain plasticity and brain-body communication. In this review, we focus on the regulatory mechanisms and functions of CRTCs in brain metabolism, lifespan, circadian rhythm and synaptic mechanisms underlying memory and emotion. We also discuss how CRTCs deregulation in cognitive and emotional disorders may provide the basis for potential clinical and therapeutic applications in neurodegenerative and psychiatric diseases.


## Trends

- CRTCs are novel regulators of neuronal circuits and brain-body communication.
- CRTC1 regulates neuroplasticity genes involved in synaptic plasticity, memory and emotional processes.
- CRTC1 dysfunction is linked to psychiatric and age-related neurodegenerative diseases.
- CRTCs are novel potential genetic factors and therapeutic targets for psychiatric and neurodegenerative diseases.

*"Mens sana in corpore sano"* (Satire X, Decimus Iūnius Iuvenālis), assumes that healthy mind exists in a healthy body, whereas harmful habits or diseases impact negatively on mind and brain function. Brain-body communication is vital for health status and when disrupted, especially in chronic degenerative or psychiatric illnesses, it influences negatively on body function.

**CRTCs: novel transcriptional players of multiple biological functions**

The brain is an exceptional regulator of body homeostasis due to its ability to sense, coordinate and respond to changes in the environment. How the brain regulates a variety of molecular signals that modulate brain-body communication (e.g., hormones, neurotransmitters…) is not fully understood. In the past decade, CRTC-dependent transcriptional control of gene expression has emerged as a central regulatory mechanism of cell and tissue function, whereas its deregulation is involved in cancer as well as immunological and age-related metabolic disorders [1]. Discovered as transcriptional regulators of **CREB** (see Glossary), CRTCs' function is broader than initially expected. CRTCs regulate not only metabolic homeostasis in peripheral tissues but also maintain body energy balance by acting directly in the brain. Thus, CRTCs are unique signaling factors that act directly on brain function but also on brain-body communication by linking energy sensing and environmental signals with metabolism, circadian rhythm and synaptic function (**Figure 1, Key Figure**). In addition, CRTC1 connects synaptic activity with CREB-dependent transcription, a synapse-to-nucleus signaling process involved in neuronal development, survival, plasticity and regeneration [2, 3]. The diverse cellular actions of CRTCs contribute not only to regulate essential body functions but also brain physiology, including homeostasis, cognition and emotion. This review summarizes the most recent findings of CRTCs' function in the brain, emphasizing their biology and regulatory mechanisms, their function on brain physiology, including metabolism, circadian rhythm, **synaptic plasticity**, memory and behavior. Finally, we discuss recent findings involving CRTCs in brain pathology, especially in psychiatric and neurodegenerative disorders, which eventually could lead to novel therapeutic avenues in age-related brain diseases.

**CRTCs: structure and regulatory mechanisms**

CREB is a ubiquitous transcription factor that plays pleiotropic roles in the nervous system. It binds to conserved cAMP-response element (CRE) promoter consensus sequences of >5000 target genes as a homodimer or as heterodimers with other members of the CREB/ATF family of transcription factors [4, 5]. Multiple signaling pathways stimulate gene expression through CREB phosphorylation at Ser133, and the resulting recruitment of **CBP/p300 coactivators**, which activate transcription by acetylating nucleosomal histones and by interacting with factors of the general transcription machinery [6, 7]. CREB phosphorylation has thus long been considered as the critical event reflecting the activation of CRE-mediated transcription. However, several lines of evidence challenged this model and suggested that CREB can be activated independently of Ser133 phosphorylation [8-10], which led to the discovery of the CRTC family of coactivators [11, 12]. The CRTC family comprises three members in mammals, with human CRTC2 and -3 sharing 32% amino acids identity with CRTC1 [12]. CRTC2 and CRTC3 are expressed in most tissues, whereas CRTC1 is mainly found in the brain [13-15]. CRTCs share a rather similar modular structure consisting of an N-terminal coil-coil CREB-binding domain, a central region containing several regulatory features, and a 200-amino acid-long C-terminal transactivation domain (**Figure 2A**). Unlike the CBP/p300 coactivators that interact with phosphorylated Ser133 in the kinase-inducible domain (KID) of CREB, CRTCs bind to its bZIP domain, which promotes CREB dimerization and DNA-binding. CRTCs are thus able to activate CREB independently of its phosphorylation status.

Importantly, CRTCs do not constitutively activate CREB, as their subcellular localization is regulated by their phosphorylation state [16, 17]. In basal conditions, they are phosphorylated and sequestered in the cytoplasm by scaffolding 14-3-3 proteins and their nuclear translocation requires the concomitant activation of calcium and cAMP signaling pathways. Increase of intracellular calcium triggers CRTCs' dephosphorylation by the $Ca^{2+}$-dependent protein phosphatase 2B (PP2B)/calcineurin, whereas cAMP-activated PKA inhibits members of the **AMPK family** of Ser/Thr kinases (including SIKs, MARKs, and AMPK itself) that phosphorylate CRTCs, leading to their dissociation from 14-3-3 proteins and subsequent nuclear translocation (**Figure 2B**). In neurons, activity-dependent nuclear import of dendritic CRTC1 is critical for the transcription-dependent phase of neuronal plasticity, although the underlying cellular and molecular regulatory mechanisms of its synapse-to-nucleus translocation are not completely understood [13, 14, 18-24].

For instance, it is still debated whether PKA-mediated inhibition of SIKs is initially required for CRTC1 cytoplasmic release and nuclear translocation or whether SIKs inhibition prevents the rephosphorylation of nuclear CRTC1, thereby increasing its persistence in the nucleus [20]. Mass spectrometry analysis of CRTC1 revealed more than 10 Ser/Thr phosphorylation sites [22, 23], among which Ser151 was initially described as the main determinant of its subcellular localization [25]. However, calcineurin-mediated dephosphorylation of Ser151 is not always sufficient to induce nuclear translocation, thus suggesting the existence of additional regulatory phosphorylation sites. The number and identity of these sites were not clearly established until the recent emergence of a consensual model implicating the dephosphorylation of three conserved serine residues: Ser64, Ser151, and Ser245 [23]. These phosphorylation sites seem to confer a specific intensity of 14-3-3 binding to each of the CRTC isoforms [26]. In addition to being phosphorylated, CRTCs are regulated by other post-translational modifications including acetylation, ubiquitination, glycosylation, and methylation [1, 4]. Nonetheless, these additional post-translational modifications have been mainly reported for CRTC2 in peripheral tissues, and their potential effects on CRTC's functions in the nervous system remain as yet unclear. Moreover, accumulating evidence also suggests that CRTCs have broader roles beyond CREB-mediated transcription (**Box 1**), but their functional significance in the brain remains unknown.

**CRTCs regulate energy balance and lifespan**

The brain depends on glucose as its main source of energy, so glucose metabolism is crucial for brain function whereas its disruption is involved in the pathophysiology of brain disorders [27]. The brain, in turn, acts as a central regulator of body metabolism by controlling hormone and neuropeptides levels during appetite, nutrient intake and satiety. In this scenario, CRTCs have recently emerged as key molecules orchestrating gene changes regulating glucose and lipid homeostasis in peripheral tissues, including the liver, muscle, adipose tissue and pancreas [[4], for a recent review]. In the peripheral nervous system, CRTCs act as regulators of adaptive responses to exercise by integrating adrenergic and cholinergic signaling in sympathetic neurons and muscle. Indeed, catecholamines released from sympathetic neurons activate CRTC2/3/CREB-mediated transcription inducing glycogen and triglyceride catabolism in the skeletal muscle during intense exercise [28]. In the central nervous system, CRTCs coordinate energy balance by sensing and

responding to hormonal and nutrient signals (**Figure 1**). Adipocyte-derived leptin promotes satiety by activating leptin receptors in the arcuate nucleus of the hypothalamus. Leptin receptors induce Ser151 dephosphorylation and nuclear translocation of CRTC1 promoting CREB-dependent expression of the satiety neuropeptide cocaine- and amphetamine-regulated transcript (*Cartpt*) [25]. Indeed, *Crtc1*$^{-/-}$ mice show a leptin-resistant obese phenotype caused by increased appetite and food intake [25, 29]. Intriguingly, hyperphagic behavior and rapidly developing obesity appear only in *Crtc1*$^{-/-}$ males, whereas mutant females exhibit mild late-onset obesity without hyperphagia [30]. Furthermore, the male-specific hyperphagia is restricted to the resting (diurnal) phase of the light cycle and accompanied by a higher diurnal locomotor activity, thus suggesting that CRTC1 plays a gender-specific role in the central control of energy balance and circadian locomotor activity. Besides, feeding inhibits AMPK leading to CRTC2-dependent activation of *Insulin receptor substrate 2* (*Irs2*) and ***corticotropin-releasing hormone* (*Crh*)** genes in the hypothalamus [31], a result that contrasts with CRTC2 inactivation in response to feeding in the liver [32]. Nonetheless, CRTC2 is not able to compensate for loss of CRTC1 function in *Crtc1*$^{-/-}$ mice suggesting that the gene targets and/or physiological functions of CRTC family members may differ.

Regulation of specific CRTCs by nutrient signals in the brain depends on the cellular and physiological contexts. In *Drosophila*, brain CRTC regulates energy balance during starvation by stimulating CREB target genes, including the short neuropeptide F (sNPF), an orthologue of mammalian neuropeptide Y implicated in maintenance of lipid and glycogen stores [33]. During re-feeding, however, insulin inhibits CRTC activity by activating SIK2 in the brain [34]. Food deprivation induces nuclear accumulation and activation of CRTC in *Drosophila* neurons, which mediates appetitive long-term memory [35]. These studies indicate that CRTC family members regulate expression of genes involved in brain responses to nutrient sensing.

Since CRTCs regulate energy homeostasis in response to nutrient signals, it is plausible that CRTCs modulate aging and longevity. Indeed, recent studies demonstrate a tight association between energy metabolism and aging, and several cellular pathways, including, among others, the mechanistic target of rapamycin (mTOR), AMPK and sirtuins, which mediate the effect of reduced energy intake on lifespan extension in lower organisms and mammals [36]. In *C. elegans*, activation of AMPK and calcineurin promotes longevity by inactivating the CRTC homologue CRTC-1 [37, 38]. Selective CRTC-1 activation in neurons is sufficient to suppress the effects of AMPK on systemic metabolism

and longevity acting through a cell-nonautonomous mechanism via regulation of catecholamine neurotransmission [38]. Notably, decreasing CRTC-1 expression extends lifespan, whereas inactivation of the CREB homologue *crh-1* precludes lifespan extension caused by mutant *CRTC-1* suggesting a CREB-mediated effect [37]. Neuronal *CRTC-1* mediates the effect of AMPK in longevity by modulating expression of genes involved in peripheral mitochondrial metabolism and dynamics, including genes regulated by the PPARα ortholog NHR-49 [38]. In contrast to these results, *CRTC* mutant flies live shorter and show reduced triglyceride and glycogen stores in response to starvation and oxidative stress, a phenotype reversed by overexpressing SIK or CRTC in neurons [34, 39]. In mice, activation of CRTC1/CREB signaling by the pain receptor transient receptor potential cation channel subfamily V member 1 (TRPV1) in dorsal root ganglia sensory neurons limits lifespan by increasing expression and secretion of the calcitonin gene-related peptide (CGRP), a molecule that restricts pancreatic insulin secretion and metabolic health in aging [40]. Together, these data suggest that neuronal CRTC regulates the crosstalk between metabolism and lifespan, although the neuronal subpopulations and CRTC-dependent gene programs involved in aging and longevity still require further investigation, especially in mammals.

The circadian clock regulates rhythmic processes to maintain physiology, metabolism and behavior during day/night cycles. Several studies have highlighted the importance of CREB in regulating genes mediating circadian clock rhythms [2]. Interestingly, CRTC1 and CRTC2 are highly expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus, the master circadian clock region. CRTC1, but not CRTC2, is increased in the middle of the day and decreased during the night in the mouse [41]. A light stimulus during the night period induces CRTC1 nuclear translocation and CREB transcriptional activation of *period1 (Per1)* and *Sik1*, the clock genes that synchronize the phase of circadian rhythms to body metabolism and behavior. In turn, SIK1-mediated CRTC1 phosphorylation decreases *Per1* in cellular circadian clock models and phase-shifting responses to light at circadian times (CT) 16-22 in mice, indicating that SIK1 suppresses the effects of light on the circadian rhythm entrainment by inactivating CREB/CRTC1 signaling [42]. It will be important in the future to discern the potential of modulating CRTC1 function to improve metabolic states and circadian rhythms in sleep and circadian clock-related diseases.

**CRTCs in synapse morphology, function and plasticity**

Throughout life, the brain constantly reorganizes synaptic connections in response to environmental stimuli, experiences, and body signals. Long-lasting forms of neuronal plasticity are thought to depend on synapse-to-nucleus signaling and subsequent gene expression related to dendritic morphology, spine formation and synapse development [2]. Accordingly, CREB-dependent transcription of neuroplasticity genes is crucial for intrinsic and synaptic plasticity [3, 6, 43], and accumulating evidence also supports CRTC1's involvement in neuronal plasticity processes, such as the maintenance of hippocampal late-phase **LTP** [13, 18, 44]. Late-LTP-eliciting stimuli and long-term memory paradigms trigger CRTC1 dephosphorylation and nuclear translocation [18, 21, 22, 24]. Indeed, synaptic activity induces CRTC1 translocation from synapses to the nucleus triggering CREB-dependent gene transcription in neurons [20]. Activity-dependent transport of CRTC1 from dendritic spines to the nucleus of cultured rat hippocampal excitatory neurons requires local elevations of calcium triggered by activation of glutamate receptors and L-type voltage-gated calcium channels. $Ca^{2+}$/calcineurin-mediated dephosphorylation of CRTC1 at serines 64, 151, and 245 induces its dissociation from 14-3-3 proteins and migration from distal stimulated synapses to the nucleus through a mechanism involving the microtubule-based retrograde motor protein dynein and a conserved arginine-rich nuclear localization signal, independently of the classical importin $\alpha/\beta$1-mediated pathway [23, 45]. In these neuronal cultures, elevations of intracellular cAMP and activation of PKA are not required for the initial translocation of CRTC1 to the nucleus, but rather they increase the persistence of nuclear CRTC1 by inactivating SIKs and thus preventing its rephosphorylation [20]. In the nucleus, CRTC1 upregulates the expression of a subset of CREB target genes that includes, among others, the neurotrophic factor *Bdnf*, the immediate-early genes *c-fos*, *Zif268*/*Egr1*, and *Arc*, the orphan nuclear receptors *Nr4a1* and *Nr4a2*, and the brain-specific growth factor *Fgf1* [18, 20-24, 44, 46-48]. Interestingly, all these genes have been involved in synaptic plasticity and memory formation. In agreement with the induction of these neuroplasticity genes, CRTC1-mediated gene transcription is required for activity- and BDNF-induced dendritic growth of developing cortical neurons [14, 19, 49]. More specifically, overexpressing CRTC1 in cultured rat cortical neurons increases basal as well as activity-induced dendrite length, whereas the downregulation of CRTC1 with a dominant-negative or by RNA silencing significantly reduced dendritic growth in cultured neurons and in the developing rat somatosensory cortex [8]. Similarly, BDNF-induced dendritic growth of rat cortical neurons requires CRTC1 nuclear translocation

resulting from activation of N-methyl-D-aspartate (NMDA) receptors by glutamate [13]. In summary, synapse-to-nucleus transport of CRTC1 is important to inform the nucleus about synaptic activity and induce the expression of neuroplasticity genes, whose products mediate plastic changes in dendrites and spines, thereby strengthening existing synaptic connections and facilitating the formation of new synapses.

**CRTCs in learning and memory**

It is well established that CREB controls gene expression programs essential for long-term synaptic plasticity and memory in a variety of organisms [6, 43, 50, 51], so acting as its transcriptional coactivators CRTCs are expected to modulate learning and/or memory. In *Drosophila,* CRTC is activated in mushroom body neurons by food deprivation mediating aversive and appetitive memory, whereas CRTC induces long-term memory and extends memory extinction [35, 52]. The transition from memory formation to maintenance is mediated by histone acetylation and shifting the transcriptional complex from CREB/CBP to CREB/CRTC [52]. Consistent with its role in CREB signaling, CRTC1 also mediates hippocampal-dependent long-term memory in mammals. Spatial and context associative learning induce CRTC1 activation in the hippocampus in a time- and stimulus-dependent manner [21, 22, 24, 44]. The regulatory mechanism of CRTC1 activation during learning involves calcineurin-dependent CRTC1 dephosphorylation and nuclear translocation in neurons of the hippocampus. Following associative learning, a complex containing phosphorylated CREB, CBP and CRTC1 is recruited to CRE-containing promoters that together with histone H3K14 acetylation induces transcription of synaptic plasticity and memory-related genes, such as *Bdnf*, *c-fos*, *Nr4a1*, *Nr4a2* and *Fgf1* [24, 44]. CRTC1 sustains memory formation as revealed by memory enhancement in mice overexpressing wild-type or constitutively active S151A/S245A or S151A/S167A mutants [22, 24, 44, 53]. Additionally, CRTC1 inactivation in the hippocampus impairs hippocampal LTP and long-term contextual fear memory, a phenotype prevented by overexpressing CRTC1 but not a cytosolic CRTC1 mutant, indicating that CRTC1 nuclear translocation is essential for memory consolidation [44]. In agreement with a CREB-dependent mechanism, disruption of CREB/CRTC1 association impairs CREB-dependent transcription, synaptic plasticity and long-term memory [49]. Although regulation of neuroplasticity genes is critical for CRTC1's function in synaptic plasticity and memory, it is unclear whether CRTC1 mediates

long-term memory by regulating synapse-to-nucleus signaling, synaptic plasticity and/or unknown pre- or post-synaptic mechanisms. A more precise understanding of the cellular and molecular mechanisms by which CRTCs regulate synaptic function and plasticity is thus critical to better discern their implication in memory.

**CRTC1 in neurodegenerative diseases**

CREB signaling dysfunction is involved in degeneration and memory deficits in neurodegenerative diseases [54]. In agreement with its role in CREB signaling, CRTC mediates neuroprotection in neurodegenerative diseases. In Huntington's disease (HD), dominant inherited mutations in the *huntingtin* (*Htt*) gene cause death of striatal medium spiny neurons leading to motor alterations. Notably, CRTC1 is reduced in the striatum of HD [55], and mutant Htt inactivates CRTC1, which likely contribute to neurodegeneration by repressing BDNF expression [56]. The Sirt1 deacetylase mediates neuroprotection and ameliorates motor deficits by activating CRTC1 in transgenic HD mouse models [56]. CRTC1 inactivation in the striatum of Htt transgenic mice induces neurodegeneration, whereas CRTC1 overexpression reduces mitochondrial toxicity by increasing PGC-1α expression [55]. Besides its role in neurodegeneration, decreased expression of CREB/CBP/CRTC1 target genes in the hippocampus is associated with long-term memory deficits in HD mice [57], which suggests a possible link between CRTC1 dysfunction and memory deficits in HD.

Parkinson´s disease (PD) is caused by dysfunction and loss of dopaminergic neurons in the substantia nigra pars compacta, which leads to motor impairments. It has been shown that neuronal p21-activated kinase 4 (PAK4) protects from neurodegeneration and motor dysfunction caused by 6-hydroxydopamine (6-OHDA) and α-synuclein in PD rat models [58]. PAK4 mediates neuroprotection by phosphorylating CRTC1 at Ser215 and inducing the expression of CREB target genes, including *Bcl-2*, *Bdnf*, and *Pgc-1α*. Levels of active pCRTC1 Ser215, but not those of total CRTC1, are significantly reduced in the substantia nigra of PD patients, as well as after 6-OHDA treatment or α-synuclein overexpression in rats [58]. These results indicate that CRTC1 phosphorylation at Ser215 is critical for PAK4-neuroprotection, although it is still unclear whether this mechanism is involved in the etiopathogenesis of PD or other neurodegenerative diseases.

Alzheimer's disease (AD), the main cause of dementia worldwide, is characterized by degeneration of neurons and synapses, and abnormal accumulation of **β-amyloid (Aβ)** peptides and phosphorylated tau in memory circuits. Deregulation of CREB/CRTC1 signaling was recently reported in brains of AD patients and mice. Thus, a CRTC1/CREB-dependent gene transcriptional program related with synapse function and plasticity is deregulated at early pathological and cognitive stages in $APP_{Sw,Ind}$ transgenic mice [21]. Impaired CRTC1 function coincides with intraneuronal Aβ accumulation suggesting that aggregated pre-plaque Aβ may perhaps lead to CRTC1 inactivation [21, 59]. The mechanism by which Aβ mediates CRTC1 inactivation involves impaired $Ca^{2+}$/calcineurin-mediated CRTC1 dephosphorylation and reduced nuclear translocation and occupancy of CREB target gene promoters [21, 46], which eventually leads to downregulation of neuroplasticity, memory and neuroprotection genes (**Figure 3**). In addition, reduced calcium influx or mobilization from intracellular stores might be responsible for disruption of CRTC1 transcriptional function caused by loss of presenilin function, as observed in a neurodegeneration mouse model [24]. Importantly, CRTC1 overexpression using a gene therapy approach ameliorates early transcriptional and memory impairments in AD and neurodegeneration mouse models, suggesting that increasing CRTC1 activity may be beneficial against cognitive decline in AD [21, 24]. Nevertheless, epileptic drugs induce CRTC1 nuclear translocation in the hippocampus, indicating that CRTC1 overactivation caused by aberrant excitability may be counterproductive [60]. Notably, CRTC1 protein and mRNA are reduced at moderate stages of AD [21, 61]. *CRTC1* promoter methylation inversely correlates with phosphorylated tau accumulation but not amyloid deposition, suggesting a possible link between tau pathology and *CRTC1* deregulation [61]. Future studies will be necessary to discern whether CRTC1 contributes to cognitive impairments and pathological hallmarks in AD and other dementing disorders.

**CRTCs dysregulation in psychiatric disorders**

Altered neuronal plasticity and cognitive impairments are common pathophysiological features of many psychiatric disorders [62]. By affecting neuroplasticity at several levels, chronic stress strongly contributes to the development of psychiatric illnesses [63]. Notably, CREB and BDNF are critically

involved in these processes [64, 65]. Consistent with CRTCs' role in CREB signaling and *Bdnf* gene expression, emerging evidence suggests that CRTCs dysregulation may be implicated in the etiopathogenesis of psychiatric disorders, such as addiction, anxiety and mood disorders. There is compelling evidence of HPA axis dysfunction in stress-related psychiatric disorders. **CRF** produced in the hypothalamic paraventricular nucleus (PVN) is the main regulator of HPA axis activity during stress, and tightly controlled CRF levels are required for a proper stress response. *Crf* transcription depends on CRTC2 and CRTC3 in rat and mouse PVN [66, 67]. Given the strong evolutionary conservation of the HPA axis regulation, one can reasonably speculate that altered levels or activity of CRTCs in human PVN might impinge on HPA axis function, and possibly participate in the development of psychiatric disorders.

Drug addiction is considered to be a disorder of neuroplasticity caused by cellular and molecular alterations in brain reward systems. Rats with a history of extended cocaine access show an escalation of cocaine consumption triggered by neuroplastic changes in the striatum. Interestingly, only a vulnerable fraction of human cocaine users or rats with extended cocaine access (~15%) loses control over intake and develops compulsive drug-seeking behaviors. The large majority of rats not exhibiting addiction-like behaviors are protected by a mechanism involving microRNA-212-enhanced Raf1 activity, resulting in adenylyl cyclase sensitization, and increased CREB phosphorylation and CRTC1 levels in the dorsal striatum [68]. Lentivirus-mediated overexpression of CRTC1 in dorsal striatal neurons indeed protects rats with extended cocaine access from escalating their cocaine consumption. Although this interesting finding awaits further confirmation by other studies, it suggests that this coactivator might play a key role in determining vulnerability to drug addiction.

Depressive and bipolar disorders, collectively referred to as mood disorders, are the most common forms of psychiatric disorders and, according to World Health Organization, the leading cause of disability worldwide. Although several classes of antidepressants are available, a substantial percentage of patients are unresponsive to these medications, calling for a better understanding of the neurobiology of depression. Increasing evidence suggests that brain neuroplasticity is disrupted in mood disorders, which is supported by the involvement of CREB and BDNF in major depressive disorder and rodent models of depression [63, 64, 69, 70]. In keeping with CRTC1's role in neuronal plasticity processes, *Crtc1*$^{-/-}$ mice exhibit depressive-like behaviors and neurobehavioral

endophenotypes related to mood disorders [39]. Concomitantly, the expression of several susceptibility genes, including *Bdnf*, its receptor *TrkB*, the nuclear receptors *Nr4a1-3*, and several other CREB-regulated genes is decreased in the prefrontal cortex and hippocampus, which suggests an altered neuroplasticity in these structures. Moreover, mutant mice display a blunted response to classical antidepressants [47, 71], which indicates that CRTC1 is required for a normal antidepressant response in mice. Interestingly, the HDAC inhibitor **SAHA** improves the depressive-like behavior of *Crtc1*$^{-/-}$ mice and restores *Bdnf* expression in their prefrontal cortex, which suggests that an epigenetic approach rescuing CRTC1-mediated neuroplasticity gene expression could be a useful antidepressant strategy [71]. A cDNA microarray study attempting to identify altered gene expression in the cortex of *Crtc1*$^{-/-}$ mice revealed an upregulation of agmatinase, the enzyme degrading the arginine-decarboxylation product agmatine [72]. Accumulating evidence implicates the agmatinergic system in brain pathology, notably in mood disorders, since post-mortem studies have shown increased agmatinase levels in brain tissues from depressed individuals [73, 74]. Acute and chronic stress decrease agmatine levels in rodent forebrain and exogenous agmatine has rapid antidepressant effect in animal models of depression. Hence, the depressive-like phenotype of *Crtc1*$^{-/-}$ mice is improved by acute agmatine treatment, which supports a causal role of the deregulated agmatinergic system in this mouse model of depression, as it was suggested for human depression [72, 74]. The relationship between CRTC1 and agmatinase regulation is still elusive and merits further investigation, as it should provide further insight into their role in psychiatric disorders, notably in human major depression. A recent study did not show a genetic association between *CRTC1* polymorphisms and major depressive disorder [75]. Nonetheless, in keeping with the shared etiological pathways of psychiatric disorders and obesity, *CRTC1* polymorphisms play a role with obesity markers in psychiatric cohorts and individuals with major depression [75, 76]. The absence of direct genetic association between *CRTC1* and depression in these studies does not however rule out the possibility that other *CRTC1* genetic variants might be associated with mood disorders or other psychiatric disorders.

## Concluding Remarks

Compelling evidence that CRTCs play a crucial role in brain plasticity has accumulated over the past ten years. The recent progress described above indicates that activity-dependent synapto-nuclear

transport of CRTC1 is instrumental in conveying information from remote dendritic post-synaptic compartments to the nucleus, where it increases the expression of CREB target neuroplasticity genes. Acting as important sensors and integrators of hormonal, metabolic, and neural signals, CRTCs regulate essential brain functions and many aspects of reciprocal brain-body communication (**Table 1**). However, many features of CRTCs' brain function and regulation remain elusive (see Outstanding Questions). For instance, CRTCs play crucial roles in brain plasticity and brain-body communication by regulating the expression of CREB target genes, but CRTCs' functions dependent or independent of gene transcription at synapses or other neuronal compartments require further investigation. Future studies aiming at further understanding these mechanisms should lead to better insight into the possible involvement of CRTCs' deregulation in cognitive, neurodegenerative and psychiatric disorders. Ultimately, it is tantalizing to envision the future development of novel treatment strategies targeting the CRTC signaling pathway.

**Acknowledgements**

The authors would like to thank present and past members of Saura´s and Cardinaux´s labs for their scientific contribution to the content of this paper. We sincerely thank Dr. A. Parra-Damas for his contribution to the figures. We thank the Library of Science and Medical Illustrations for providing free access and publication of some images used in the figures. This study was supported by grants from the Ministerio de Economía, Industria y Competitividad and Instituto Carlos III of Spain (SAF2016-80027-R, CIBERNED CB06/05/0042), Generalitat de Catalunya (2014 SGR0984) and the Alzheimer´s disease research program of the BrightFocus Foundation (Ref. A2014417S) to C.A.S., and by grants from the Swiss National Science Foundation (31003A-135692 and 31003A-170126) to J-R.C.

**Glossary**

**AMPK family:** the AMP-activated protein kinase (AMPK) is an important energy sensor of intracellular ATP levels. It is activated when AMP levels increase in response to a lowering of ATP levels. Other members of the AMPK family include the salt-inducible kinases (SIKs) and microtubule affinity regulating kinases (MARKs), whose activity is not regulated by AMP levels.

**β-amyloid (Aβ):** 39-43 amino acid-long peptides generated by sequential cleavage of the amyloid precursor protein (APP). β-amyloid is accumulated in amyloid plaques in the brain of Alzheimer´s disease patients. Abnormal accumulation, aggregation and deposition of β-amyloid are thought to contribute to degeneration of neurons in Alzheimer´s disease.

**CBP/p300 coactivators:** CREB-binding protein (CBP) and p300 are closely related transcriptional coactivators that interact with numerous transcription factors. They activate transcription through their intrinsic histone acetyltransferase activity and by helping to recruit components of the general transcriptional machinery to gene promoters.

**CREB:** the cAMP-response element (CRE) binding protein (CREB) is a 43 kDa transcription factor that binds to the highly conserved palindromic CRE consensus sequence 5´-TGACGTCA-3´ of target gene promoters. CREB belongs to the bZIP superfamily of transcription factors that are characterized by a leucine zipper domain that mediates dimerization and a C-terminal basic region that binds to the DNA. Several cellular signals converge to activate CREB-mediated gene transcription into the nucleus.

**CRH or CRF:** corticotropin-releasing hormone (CRH), also called corticotropin-releasing factor (CRF), is a 41-amino acid peptide that is the primary regulator of the mammalian stress response. CRH is secreted by the paraventricular nucleus of the hypothalamus in response to stress and activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to a release of glucocorticoids from the adrenal gland cortex.

**SAHA:** Suberoylanilide hydroxamic acid (also known as vorinostat) is a compound that inhibits class I and II histone deacetylases (HDACs). Systemic administration of SAHA increases histone acetylation in the brain and has antidepressant effects in mice.

**Synaptic plasticity and LTP:** Synaptic plasticity refers to structural and functional modifications of synapses resulting in changes in synaptic efficacy. One cellular form of synaptic plasticity is long-term potentiation (LTP), which involves persistent strengthening of synaptic response following high-frequency stimulation. Long-lasting synaptic plasticity mediated by neuronal activity requires transcription of gene programs. Importantly, neuronal plastic changes that persist for days or longer as a result of learning and training induce memory formation.

**Box 1. Emerging roles for CRTCs beyond CREB coactivation**

There is growing evidence that CRTCs' functions are not restricted to CREB coactivation. CRTC1 has the capacity to bind to the bZIP domain of c-Fos and c-Jun, forming the AP-1 transcription factor, thereby promoting cell proliferation and transformation [77]. Given the importance of Fos family members (c-Fos, FosB, ΔFosB) in brain plasticity processes, it remains to determine whether CRTC1 is also coactivating AP-1-mediated transcription in the brain. Linking endoplasmic reticulum (ER) stress and fasting gluconeogenesis, CRTC2 interacts with the bZIP transcription factor ATF6, which results in a decrease of CREB-mediated gluconeogenic genes expression [78]. Further suggesting a more general role in transcription beyond CREB, it was recently shown that CRTC2 modulates the transcriptional activity of the glucocorticoid and progesterone receptors in liver cells [79], thus raising the interesting possibility that CRTCs might act as coactivators of the nuclear receptor superfamily in various tissues, including the brain.

Besides their transcriptional coactivator function, CRTCs have additional roles in RNA splicing regulation and ER-Golgi trafficking. Depending on the cell type or promoter context, CRTCs coordinately or independently regulate transcription and alternative pre-mRNA splicing of CRE-containing genes, such as for instance *Nr4a2* [80]. CRTC1-dependent alternative splicing is mediated by a functional domain distinct from the C-terminal transactivation domain (**Figure 2A**). In addition to these expanding new functions, CRTC2 has been shown to play a role in hepatic lipid metabolism by regulating vesicle trafficking from the ER to the Golgi and the resulting processing of Sterol Regulatory Element-Binding Protein 1 [81]. Taken together, these emerging new roles of CRTCs beyond CREB coactivation highlight the diversity of the cellular processes involving these coactivators. Future studies should provide further insight into their potential importance in the brain.

**Outstanding Questions**

The localization of CRTC1 at different neuronal compartments, such as cytosol, nucleus, dendrites and synapses, raises the possibility that CRTC1 may be involved in multiple functions depending on its subcellular location. Why CRTC1 is located at synapses? Does CRTC1 play a role at synapses independently of its transcriptional function? Is CRTC1 function at synapses dependent or independent of its transcriptional activity?

CRTC1 regulates functional and structural synaptic plasticity in the hippocampus. Which are the specific molecular mechanisms regulated by CRTC1 during synaptic plasticity? Does CRTC1 regulate presynaptic or postsynaptic mechanisms during synaptic plasticity? Does CRTC1-dependent synaptic plasticity mediate memory encoding or storage?

Deregulation of CRTC1 occurs in several neurodegenerative and psychiatric disorders. Is there a common mechanism leading to disruption of CRTC1/CREB pathway in these disorders? Is CRTC1 affecting specific or common pathological hallmarks in these brain disorders? Are deregulated CRTCs perturbing adult hippocampal neurogenesis that is involved in memory, cognitive function and mood regulation? Importantly, are CRTC1-based therapeutic strategies beneficial to ameliorate pathological and behavioral changes in brain diseases?

**References**


1 Escoubas, C.C.*, et al.* (2017) Deregulation of CRTCs in aging and age-related disease risk. *Trends Genet.* 33, 303-321

2 Deisseroth, K.*, et al.* (2003) Signaling from synapse to nucleus: the logic behind the mechanisms. *Curr. Opin. Neurobiol.* 13, 354-365

3 Cohen, S. and Greenberg, M.E. (2008) Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. *Annu. Rev. Cell. Dev. Biol.* 24, 183-209

4 Altarejos, J.Y. and Montminy, M. (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat. Rev. Mol. Cell. Biol.* 12, 141-151

5 Mayr, B. and Montminy, M. (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell. Biol.* 2, 599-609

6 Benito, E. and Barco, A. (2010) CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. *Trends Neurosci.* 33, 230-240

7 Lonze, B.E. and Ginty, D.D. (2002) Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35, 605-623

8 Briand, L.A.*, et al.* (2015) Serine 133 phosphorylation is not required for hippocampal CREB-mediated transcription and behavior. *Learn. Mem.* 22, 109-115

9 Brindle, P.*, et al.* (1995) Multiple protein kinase A-regulated events are required for transcriptional induction by cAMP. *Proc. Natl. Acad. Sci. U. S. A.* 92, 10521-10525

10 Impey, S.*, et al.* (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* 16, 973-982

11 Conkright, M.D.*, et al.* (2003) TORCs. Transducers of Regulated CREB Activity. *Mol Cell* 12, 413-423

12 Iourgenko, V.*, et al.* (2003) Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12147-12152

13 Kovács, K.A.*, et al.* (2007) TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. *Proc. Natl. Acad. Sci. U. S. A.* 104, 4700-4705

14 Li, S.*, et al.* (2009) TORC1 regulates activity-dependent CREB-target gene transcription and dendritic growth of developing cortical neurons. *J. Neurosci.* 29, 2334-2343

15 Watts, A.G.*, et al.* (2011) The distribution of messenger RNAs encoding the three isoforms of the transducer of regulated cAMP responsive element binding protein activity in the rat forebrain. *J. Neuroendocrinol.* 23, 754-766

16 Screaton, R.A.*, et al.* (2004) The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. *Cell* 119, 61-74

17 Bittinger, M.A.*, et al.* (2004) Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. *Curr. Biol.* 14, 2156-2161

18 Zhou, Y.*, et al.* (2006) Requirement of TORC1 for late-phase long-term potentiation in the hippocampus. *PLoS One* 1, e16

19 Finsterwald, C.*, et al.* (2010) Regulation of dendritic development by brain-derived neurotrophic factor (BDNF) requires activation of the CREB-regulated transcription coactivator 1 (CRTC1) by glutamate. *J. Biol. Chem.* 285, 28587-28595

20 Ch'ng, T.H.*, et al.* (2012) Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. *Cell* 150, 207-221

21 Parra-Damas, A.*, et al.* (2014) Crtc1 activates a transcriptional program deregulated at early Alzheimer's disease-related stages. *J. Neurosci.* 34, 5776-5787

22 Nonaka, M.*, et al.* (2014) Region-specific activation of CRTC1-CREB signaling mediates long-term fear memory. *Neuron* 84, 92-106

23 Ch'ng, T.H.*, et al.* (2015) Cell biological mechanisms of activity-dependent synapse to nucleus translocation of CRTC1 in neurons. *Front. Mol. Neurosci.* 8, 48

24 Parra-Damas, A.*, et al.* (2017) CRTC1 function during memory encoding is disrupted in neurodegeneration. *Biol. Psychiatry* 81, 111-123

25 Altarejos, J.Y., *et al.* (2008) The Creb1 coactivator Crtc1 is required for energy balance and fertility. *Nat. Med.* 14, 1112-1117

26 Sonntag, T., *et al.* (2017) Analysis of a cAMP regulated coactivator family reveals an alternative phosphorylation motif for AMPK family members. *PLoS One* 12, e0173013

27 Mergenthaler, P., *et al.* (2013) Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci.* 36, 587-597

28 Bruno, N.E., *et al.* (2014) Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. *EMBO J.* 33, 1027-1043

29 Breuillaud, L., *et al.* (2009) Mouse fertility is not dependent on the CREB coactivator Crtc1. *Nat. Med.* 15, 989-990; author reply 991

30 Rossetti, C., *et al.* Gender-specific alteration of energy balance and circadian locomotor activity in the Crtc1 knockout mouse model of depression. *Transl. Psychiatry* (in press)

31 Lerner, R.G., *et al.* (2009) A role for the CREB co-activator CRTC2 in the hypothalamic mechanisms linking glucose sensing with gene regulation. *EMBO Rep.* 10, 1175-1181

32 Koo, S.H., *et al.* (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* 437, 1109-1111

33 Shen, R., *et al.* (2016) Neuronal energy-sensing pathway promotes energy balance by modulating disease tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 113, E3307-E3314

34 Wang, B., *et al.* (2008) The insulin-regulated CREB coactivator TORC promotes stress resistance in Drosophila. *Cell Metab.* 7, 434-444

35 Hirano, Y., *et al.* (2013) Fasting launches CRTC to facilitate long-term memory formation in Drosophila. *Science* 339, 443-446

36 Finkel, T. (2015) The metabolic regulation of aging. *Nat. Med.* 21, 1416-1423

37 Mair, W., *et al.* (2011) Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. *Nature* 470, 404-408

38 Burkewitz, K.*, et al.* (2015) Neuronal CRTC-1 governs systemic mitochondrial metabolism and lifespan via a catecholamine signal. *Cell* 160, 842-855

39 Choi, S.*, et al.* (2011) Drosophila salt-inducible kinase (SIK) regulates starvation resistance through cAMP-response element-binding protein (CREB)-regulated transcription coactivator (CRTC). *J. Biol. Chem.* 286, 2658-2664

40 Riera, C.E.*, et al.* (2014) TRPV1 pain receptors regulate longevity and metabolism by neuropeptide signaling. *Cell* 157, 1023-1036

41 Sakamoto, K.*, et al.* (2013) Clock and light regulation of the CREB coactivator CRTC1 in the suprachiasmatic circadian clock. *J. Neurosci.* 33, 9021-9027

42 Jagannath, A.*, et al.* (2013) The CRTC1-SIK1 pathway regulates entrainment of the circadian clock. *Cell* 154, 1100-1111

43 Kandel, E.R. (2001) The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294, 1030-1038.

44 Uchida, S.*, et al.* (2017) CRTC1 nuclear translocation following learning modulates memory strength via exchange of chromatin remodeling complexes on the Fgf1 gene. *Cell Rep.* 18, 352-366

45 Herbst, W.A. and Martin, K.C. (2017) Regulated transport of signaling proteins from synapse to nucleus. *Curr. Opin. Neurobiol.* 45, 78-84

46 España, J.*, et al.* (2010) beta-Amyloid disrupts activity-dependent gene transcription required for memory through the CREB coactivator CRTC1. *J. Neurosci.* 30, 9402-9410

47 Breuillaud, L.*, et al.* (2012) Deletion of CREB-regulated transcription coactivator 1 induces pathological aggression, depression-related behaviors, and neuroplasticity genes dysregulation in mice. *Biol. Psychiatry* 72, 528-536

48 Fukuchi, M.*, et al.* (2015) Neuromodulatory effect of Galphas- or Galphaq-coupled G-protein-coupled receptor on NMDA receptor selectively activates the NMDA receptor/Ca2+/calcineurin/cAMP response element-binding protein-regulated transcriptional

coactivator 1 pathway to effectively induce brain-derived neurotrophic factor expression in neurons. *J. Neurosci.* 35, 5606-5624

49 Rexach, J.E.*, et al.* (2012) Dynamic O-GlcNAc modification regulates CREB-mediated gene expression and memory formation. *Nat. Chem. Biol.* 8, 253-261

50 Silva, A.J.*, et al.* (1998) CREB and memory. *Annu. Rev. Neurosci.* 21, 127-148

51 Yin, J.C. and Tully, T. (1996) CREB and the formation of long-term memory. *Curr. Opin. Neurobiol.* 6, 264-268

52 Hirano, Y.*, et al.* (2016) Shifting transcriptional machinery is required for long-term memory maintenance and modification in Drosophila mushroom bodies. *Nat. Comm.* 7, 13471

53 Sekeres, M.J.*, et al.* (2012) Increasing CRTC1 function in the dentate gyrus during memory formation or reactivation increases memory strength without compromising memory quality. *J. Neurosci.* 32, 17857-17868

54 Saura, C.A. and Valero, J. (2011) The role of CREB signaling in Alzheimer's disease and other cognitive disorders. *Rev. Neurosci.* 22, 153-169

55 Chaturvedi, R.K.*, et al.* (2012) Transducer of regulated CREB-binding proteins (TORCs) transcription and function is impaired in Huntington's disease. *Hum. Mol. Genet.* 21, 3474-3488

56 Jeong, H.*, et al.* (2012) Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nat. Med.* 18, 159-165

57 Giralt, A.*, et al.* (2012) Long-term memory deficits in Huntington's disease are associated with reduced CBP histone acetylase activity. *Hum. Mol. Genet.* 21, 1203-1216

58 Won, S.-Y.*, et al.* (2016) Nigral dopaminergic PAK4 prevents neurodegeneration in rat models of Parkinson's disease. *Sci. Transl. Med.* 8, 367ra170

59 Wilson, E.N.*, et al.* (2016) Intraneuronal amyloid beta accumulation disrupts hippocampal CRTC1-dependent gene expression and cognitive function in a rat model of Alzheimer disease. *Cereb. Cortex* 27, 1501-1511

60 Dubey, D. and Porter, B.E. (2016) CRTC1 nuclear localization in the hippocampus of the pilocarpine-induced status epilepticus model of temporal lobe epilepsy. *Neuroscience* 320, 57-68

61 Mendioroz, M.*, et al.* (2016) CRTC1 gene is differentially methylated in the human hippocampus in Alzheimer's disease. *Alzheimer's Res. Ther.* 8, 15

62 Arguello, P.A. and Gogos, J.A. (2012) Genetic and cognitive windows into circuit mechanisms of psychiatric disease. *Trends Neurosci.* 35, 3-13

63 Pittenger, C. and Duman, R.S. (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* 33, 88-109

64 Carlezon, W.A., Jr.*, et al.* (2005) The many faces of CREB. *Trends Neurosci.* 28, 436-445

65 Castren, E. and Hen, R. (2013) Neuronal plasticity and antidepressant actions. *Trends Neurosci.* 36, 259-267

66 Aguilera, G. and Liu, Y. (2012) The molecular physiology of CRH neurons. *Front. Neuroendocrinol.* 33, 67-84

67 Jurek, B.*, et al.* (2015) Oxytocin regulates stress-induced Crf gene transcription through CREB-Regulated Transcription Coactivator 3. *J. Neurosci.* 35, 12248-12260

68 Hollander, J.A.*, et al.* (2010) Striatal microRNA controls cocaine intake through CREB signalling. *Nature* 466, 197-202

69 Blendy, J.A. (2006) The role of CREB in depression and antidepressant treatment. *Biol. Psychiatry* 59, 1144-1150

70 Duman, R.S. and Monteggia, L.M. (2006) A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* 59, 1116-1127

71 Meylan, E.M.*, et al.* (2016) The HDAC inhibitor SAHA improves depressive-like behavior of CRTC1-deficient mice: Possible relevance for treatment-resistant depression. *Neuropharmacology* 107, 111-121

72 Meylan, E.M.*, et al.* (2016) Involvement of the agmatinergic system in the depressive-like phenotype of the Crtc1 knockout mouse model of depression. *Transl. Psychiatry* 6, e852

73 Laube, G. and Bernstein, H.G. (2017) Agmatine: multifunctional arginine metabolite and magic bullet in clinical neuroscience? *Biochem. J.* 474, 2619-2640

74 Neis, V.B.*, et al.* (2017) Therapeutic potential of agmatine for CNS disorders. *Neurochem. Int.* 108, 318-331

75 Quteineh, L.*, et al.* (2016) Association of CRTC1 polymorphisms with obesity markers in subjects from the general population with lifetime depression. *J. Affect. Disord.* 198, 43-49

76 Choong, E.*, et al.* (2013) Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. *JAMA Psychiatry* 70, 1011-1019

77 Canettieri, G.*, et al.* (2009) The coactivator CRTC1 promotes cell proliferation and transformation via AP-1. *Proc. Natl. Acad. Sci. U. S. A.* 106, 1445-1450

78 Wang, Y.*, et al.* (2009) The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature* 460, 534-537

79 Hill, M.J.*, et al.* (2016) CRTC2 is a coactivator of GR and couples GR and CREB in the regulation of hepatic gluconeogenesis. *Mol. Endocrinol.* 30, 104-117

80 Amelio, A.L.*, et al.* (2009) Bipartite functions of the CREB co-activators selectively direct alternative splicing or transcriptional activation. *EMBO J.* 28, 2733-2747

81 Han, J.*, et al.* (2015) The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. *Nature* 524, 243-246

**Table 1. Physiological and pathological processes involving CRTCs in the brain.**

| CRTC isoform | Organism | Cell type or brain region | Physiological process or pathology | Refs |
|---|---|---|---|---|
| CRTC1 | Mouse | Hypothalamus | Central regulation of energy balance | [25, 29, 30] |
| | Human | ? | Obesity in psychiatric cohorts | [75, 76] |
| | Mouse | Spinal cord sensory neurons | Pain, metabolic health, lifespan | [40] |
| | Mouse | Suprachiasmatic nucleus | Circadian rhythm | [41, 42] |
| | Rodents | Cortical and hippocampal neurons, hippocampus | Activity-dependent nuclear translocation, neuronal plasticity, L-LTP maintenance | [13, 14, 18-20, 23, 44, 49] |
| | Mouse | Hippocampus, amygdala | Long-term memory | [21, 22, 24, 44, 53] |
| | Human, mouse | Striatum, cortex | Huntington's disease | [55, 56] |
| | Human, rat | Substantia nigra | Parkinson's disease | [58] |
| | Human, rodents | Hippocampus | Alzheimer's disease | [21, 24, 46, 59, 61] |
| | Rat | Hippocampus | Epilepsy | [60] |
| | Rat | Striatum | Cocaine addiction | [68] |
| | Mouse | Prefrontal cortex, hippocampus | Mood disorders, treatment-resistant depression | [47, 71, 72] |
| CRTC2 | Mouse | Hypothalamus | Glucose sensing | [31] |
| | Rodents | Hypothalamus | *Crf* expression, stress response | [66, 67] |
| CRTC3 | Rodents | Hypothalamus | *Crf* expression, stress | [66, 67] |

| | | | | |
|---|---|---|---|---|
| | | | response | |
| CRTC | *Drosophila* | Brain | Central regulation of energy balance | [33, 34, 39] |
| | | | Appetitive long-term memory | [35, 52] |
| CRTC-1 | *C. elegans* | Neurons | Central regulation of metabolism and lifespan | [37, 38] |

**Figure Legends**

**Figure 1. Key Figure. Physiological functions of CRTCs in the central and peripheral nervous system.** CRTC family members modulate key physiological processes in the central and peripheral nervous system by regulating CREB-dependent gene transcription. The left image depicts the brain regions (black arrows) and target peripheral organs (blue arrows), the cellular processes and target genes (italic) or proteins (capital letters) regulated directly or indirectly by CRTCs in mammals. Light blue arrows indicate transcriptional function of CRTCs in the brain, and dark blue arrows represent CRTC action directly in peripheral nerves or organs. For instance, CRTC1 promotes expression and release of the CGRP neuropeptide in dorsal root ganglion neurons targeting insulin secretion in the pancreas, and catecholamines released from sympathetic neurons activate CRTC2/3 in skeletal muscle during intense exercise. The right picture shows the cellular and physiological processes regulated by brain CRTC in *C. elegans* and *Drosophila*. Details of central and peripheral actions of CRTCs and references are described in the text. Images were freely provided by the Library of Science and Medical Illustrations.

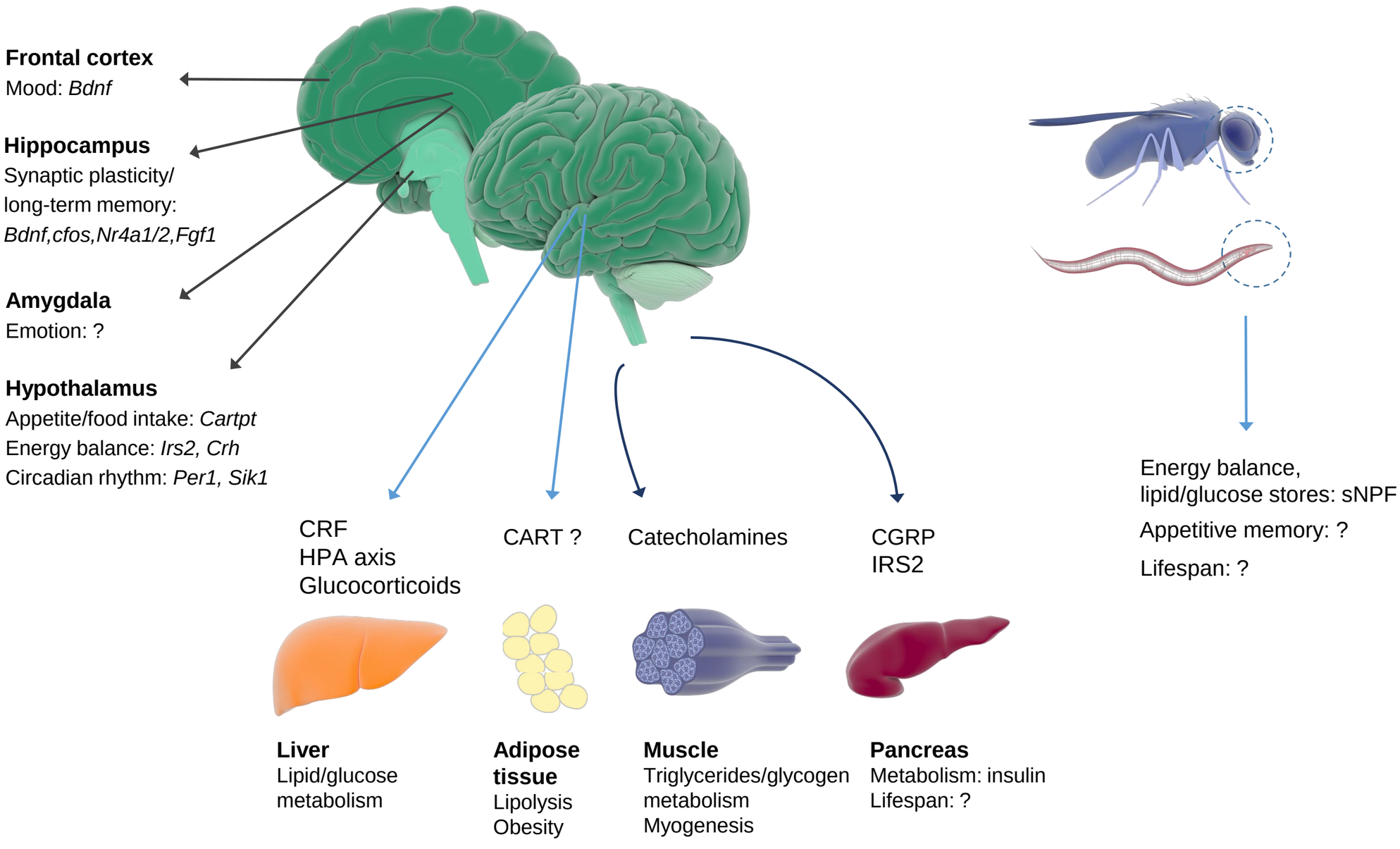
**Figure 2. Structure and regulation of CRTC1, the major brain isoform of the CRTC family.** (A) CRTC1 comprises an N-terminal CREB-binding domain (CBD) followed by a central regulatory region containing a nuclear localization sequence (NLS), two nuclear export sequences (NES1, NES2) and a splicing domain (SD) preceding the C-terminal transactivation domain (TAD). CRTC1 phosphorylation at Ser64, Ser151, and Ser245 by members of the AMPK family of Ser/Thr kinases (SIK1/2, MARK2, AMPK) promotes association with 14-3-3 proteins and cytoplasmic retention. Acetylation of several lysine residues (Lys 13, 20, 33, 178, 197) in CRTC1 has been reported [56], but its putative regulatory role is still unclear and awaiting further investigation. The numbers refer to the amino acid sequence of mouse CRTC1 protein. (B) Regulation of CRTC1 activity by $Ca^{2+}$ and cAMP signals. Neuronal activity triggers $Ca^{2+}$ influx through L-type voltage-gated calcium channels (L-VGCC), leading to calcineurin (CaN) activation and CRTC1 dephosphorylation. In addition, stimulation of adenylate cyclase (AC) by G protein-coupled receptors increases cAMP levels and activates PKA, which inhibits members of the AMPK family such as SIK. Dephosphorylated CRTC1 translocates to the nucleus and promotes expression of specific CRE-containing target genes.

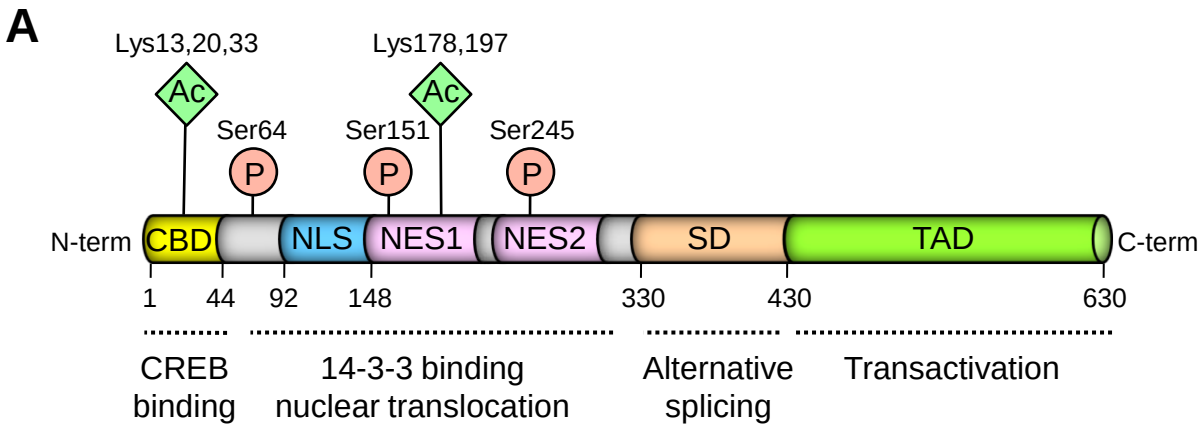
ATP: adenosine triphosphate; $Ca^{2+}$: calcium; cAMP: cyclic adenosine monophosphate; CBP: CREB-binding protein; CRE: cAMP response element; SIK: salt-inducible kinase; TBP: TATA box-binding protein.

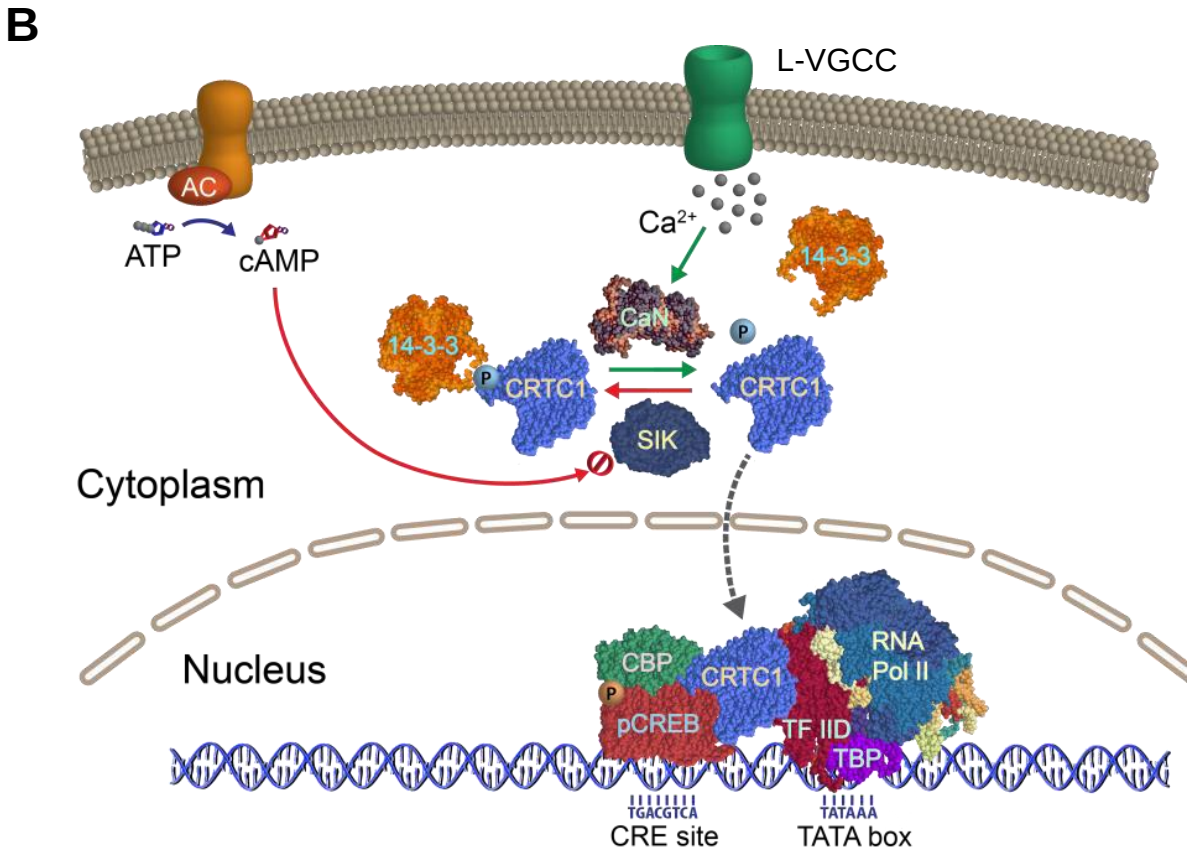
**Figure 3. Molecular mechanisms of disruption of CRTC1 signaling in Alzheimer´s disease.** Schematic cellular model depicting disruption of activity-induced CRTC1/CREB-dependent gene transcription by Aβ pathology and presenilin genes during pathological events in Alzheimer´s disease. Abnormal accumulation of intracellular and/or extracellular Aβ oligomers in neurons negatively impact on neuronal activity resulting in altered function of neurotransmitter GPCRs and L-VGCCs leading to reduced cAMP and $Ca^{2+}$ levels. The resulting impaired activation of calcineurin decreases CRTC1 dephosphorylation and nuclear translocation. On the other hand, loss of presenilin function in neurons of the postnatal forebrain is known to impair glutamate neurotransmission, which reduces intracellular $Ca^{2+}$ levels mobilized from NMDAR and ER stores. PS inactivation reduces CRTC1 dephosphorylation, nuclear translocation, and transcriptional function. The physiological effects of Aβ accumulation and PS inactivation are a disruption of CRTC1/CREB target genes in neurons.

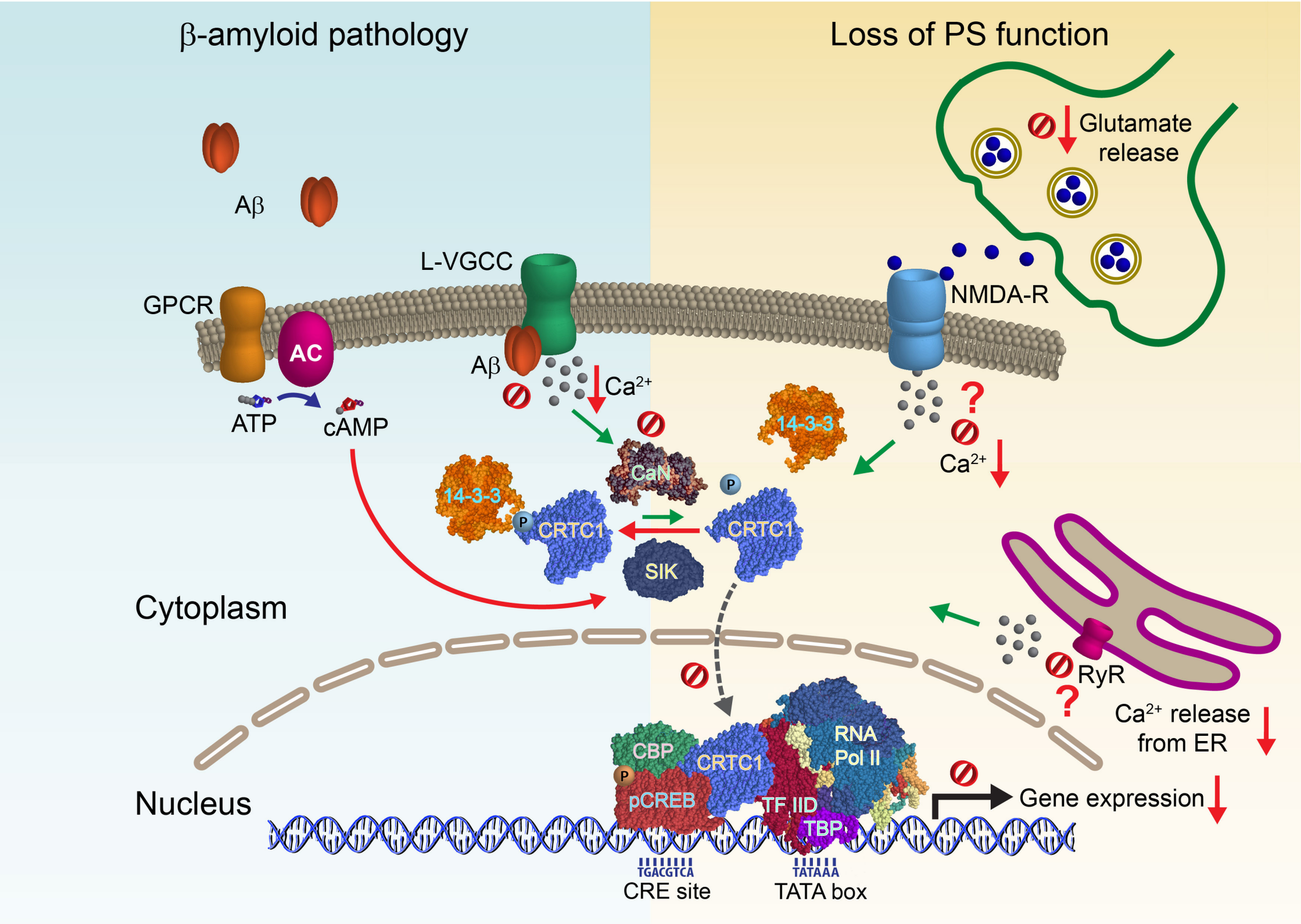
14-3-3: 14-3-3 adapter protein; Aβ: β-amyloid; AC: Adenylate cyclase; CBP: CREB binding protein; CaN: calcineurin; ER: endoplasmic reticulum; GPCR: G-protein coupled receptor; L-VGCC: L-type voltage-gated calcium channel; NMDAR: N-methyl-D-aspartate receptor; PS: presenilin; RyR: Ryanodine receptor; SIK: salt-inducible kinase.

# Physiological functions of CRTCs in the central and peripheral nervous system




A
Lys13,20,33
Lys178,197
Ac
Ac
Ser64
Ser151
Ser245
P
P
P
N-term
CBD
NLS
NES1
NES2
SD
TAD
C-term
1
44
92
148
330
430
630
CREB binding
14-3-3 binding nuclear translocation
Alternative splicing
Transactivation


B
L-VGCC
AC
ATP
cAMP
Ca2+
14-3-3
CaN
P
14-3-3
P
CRTC1
CRTC1
SIK
Cytoplasm
Nucleus
CBP
CRTC1
RNA Pol II
P
pCREB
TF IID
TBP
TGACGTCA
TATAAA
CRE site
TATA box


β-amyloid pathology
Loss of PS function
Aβ
L-VGCC
GPCR
AC
ATP
cAMP
Aβ
Ca2+
Glutamate release
NMDA-R
14-3-3
CaN
P
CRTC1
SIK
Ca2+
Cytoplasm
RyR
Ca2+ release from ER
Nucleus
CBP
CRTC1
RNA Pol II
pCREB
TF IID
TBP
Gene expression
TGACGTCA
CRE site
TATAAA
TATA box