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Function and Regulation of CREB Family Transcription Factors in the Nervous System

Review

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CREB and its close relatives are now widely accepted as prototypical stimulus-inducible transcription factors. In many cell types, these factors function as effector molecules that bring about cellular changes in response to discrete sets of instructions. In neurons, a wide range of extracellular stimuli are capable of activating CREB family members, and CREB-dependent gene expression has been implicated in complex and diverse processes ranging from development to plasticity to disease. In this review, we focus on the current level of understanding of where, when, and how CREB family members function in the nervous system.

In multicellular organisms, the ability of a cell to communicate with its environment is critical for ensuring that the cell functions appropriately within the organism. It is well established that extracellular cues are able to promote drastic changes and exert long-lasting effects such as promoting cell growth, survival or death, proliferation, and differentiation. These and other cellular processes are largely orchestrated by stimulus-induced changes in gene expression. Such stimuli exert their effects by triggering signaling cascades that ultimately converge onto nuclear transcription factors. To date, a large number of transcription factors have been identified that function in a wide variety of processes. These include but are not limited to SRF, NF-κB, Fos and Jun, C/EBP, and related factors. Among this class of gene regulatory factors, CREB stands out as the prototype as it was among the earliest identified, is among the most widely expressed, and historically has been the most highly scrutinized. In this review, we highlight the function and regulation of CREB family transcription factors as key mediators of stimulus-induced nuclear responses that underlie the development, function, and plasticity of the nervous system.

CREB Family Transcription Factors

CREB (cAMP response element binding protein) belongs to the bZIP superfamily of transcription factors, and within this superfamily, CREB and the closely related factors CREM (cAMP response element modulator) and ATF-1 (activating transcription factor 1) comprise a subcategory referred to as the CREB family. Like all bZIP transcription factors, CREB family members contain a C-terminal basic domain that mediates DNA binding, and a leucine zipper domain that facilitates dimerization. Between CREB, CREM, and ATF-1, there is a high degree of similarity, especially within the bZIP domain, which is consistent with the findings that these factors can form both homo- and heterodimers, and that each can bind to the same *cis*-regulatory element (reviewed in De Cesare et al., 1999; Mayr and Montminy, 2001; Shaywitz and Greenberg, 1999). This element, the cAMP response element (CRE), consists of the palindromic consensus sequence TGACGTCA. While many CREB binding sites are comprised of variations of this consensus motif, almost all harbor the core sequence CGTCA.

While the bZIP domain mediates DNA binding and dimerization, the remaining domains of CREB family members serve to facilitate interactions with coactivators and components of the transcriptional machinery that ultimately carry out RNA synthesis. The functional domains of CREB and its relatives (Foulkes et al., 1991; Gonzalez et al., 1989; Hai et al., 1989; Hoeffler et al., 1988) are schematically represented in Figure 1. Interestingly, both *Creb* and *Crem* encode transcription factors that, depending on the exon usage, can function as either transcriptional activators or repressors (Foulkes et al., 1991; Walker et al., 1996). The most abundant CREB isoforms, $CREB_{\alpha}$ and $CREB_{\Delta}$, contain the bZIP domain and two glutamine rich domains, referred to as Q1 and Q2/CAD (constitutive active domain) that are separated by the kinase inducible domain (KID). These two activators differ only with respect to the presence or absence of the α domain. Within the KID resides the critical residue, Ser-133, which when phosphorylated in a stimulus-inducible manner makes the KID domain a binding target for the transcriptional coactivator, CREB binding protein (CBP) (Chrivia et al., 1993; Kwok et al., 1994). CBP binds to the Ser-133-phosphorylated KID domain via its KIX domain (Parker et al., 1996; Radhakrishnan et al., 1997), and it is the stimulus-dependent interaction between these two domains that is believed to function as the trigger for inducible gene expression. Q2/CAD interacts with components of the basal transcriptional machinery and may be responsible for facilitating stimulus-independent CRE-driven gene expression (Ferreri et al., 1994; Quinn, 1993; Xing et al., 1995). The functional significance of these CREB domains and their respective interacting molecules will be considered in greater detail in a subsequent section of this review.

CREB Function

As is demonstrated by an extensive, if not daunting, body of literature, CREB is activated in response to a vast array of physiological stimuli. While the most immediate consequence of this activation is the initiation of new gene transcription, it is clear that these many stimuli elicit an equally varied host of cellular responses. What has thus remained puzzling is how such a widely expressed and readily activated transcription factor can facilitate responses so diverse and yet so specific (Figure 2). In the following section, we discuss a few of the processes relevant to the function of the nervous system in which CREB family transcription factors are believed to play an important role.

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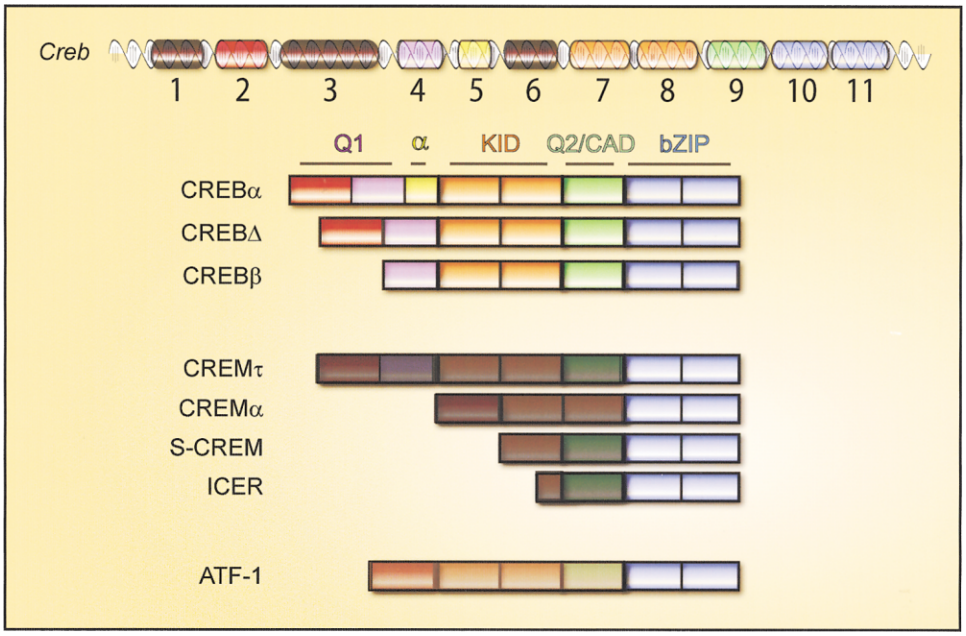

Figure 1. Gene Organization and Domain Structure of CREB Family Transcription Factors

The *Creb* and *Crem* genes support expression of multiple splice variants, while the *Atf-1* gene encodes one major protein product. $CREB_{\alpha}$ and $CREB_{\Delta}$ are the major products of the *Creb* gene. $CREB_{\beta}$, while a normally minor product, is upregulated in the $CREB^{\alpha/\Delta}$ hypomorphic mouse (see text for details). The *Crem* gene encodes both activators ($CREM_{\tau}$ and $CREM_{\alpha}$) and repressors (S-CREM and ICER) of transcription. An alternate, CRE-driven intronic promoter within the *Crem* gene drives expression of the repressor ICER in a subset of neuroendocrine tissues. *Creb* is widely expressed throughout the nervous system and elsewhere, while *Atf-1* and *Crem* exhibit more restricted patterns of expression.

CREB in Development of the Nervous System

Within the nervous system, growth factors and other stimuli regulate a wide range of processes including proliferation of neuronal precursors and the growth, survival, and synaptic connectivity of developing neurons. Many, if not all, of the stimuli believed to contribute to such processes have the capacity to signal to the nucleus to influence gene expression. The observation that CREB phosphorylation and CRE-mediated gene expression occur in response to mitogens, neurotrophins, and other neuronal growth factors has prompted an investigation of the possible contribution of this specific factor to key developmental events (Figure 3).

CREB Family Members and Neuronal Survival. Studies in which CREB family members were inhibited in neurons in vitro suggested that CREB-mediated gene expression is both necessary and sufficient for survival of multiple neuronal subtypes (Bonni et al., 1999; Riccio et al., 1999; Walton et al., 1999b). Genetic studies using *Creb* null mice (Rudolph et al., 1998) have supported these in vitro findings, and demonstrated a requirement of CREB per se in the survival of both DRG sensory neurons in vivo and sympathetic neurons in vitro (Lonze et al., 2002). CREB may exert this pro-survival effect by regulating the transcription of pro-survival factors, including *bcl-2* (Riccio et al., 1999). This finding, in conjunction with the observation that in some populations of neurons, the loss of CREB imparts a Bax-dependent form of apoptosis (Lonze et al., 2002), suggests a model in which the activation of CREB shifts the intracellular balance between survival- and death-promoting factors in favor of those that support survival.

For neurons of the CNS, survival requirements appear to extend beyond CREB alone. Recently, Schütz and colleagues examined neuronal survival in mice in which CREB function can be disrupted in a regionally and temporally specific manner (Mantamadiotis et al., 2002). Elimination of CREB in both neurons and glia of the CNS showed that neither neuronal survival nor CNS integrity is compromised upon the elimination of CREB alone. In sharp contrast, however, when eliminated in the same cell types in the absence of its family member CREM, the result is perinatal lethality, and a marked loss of neurons in several CNS regions observed at birth. This neuronal loss is not the consequence of proliferative defects, but rather results from an increase in apoptosis that begins during mid-gestation. Further, when CREB is eliminated beginning postnatally in a *Crem* null background, the result is a profound degeneration of the CNS that occurs progressively throughout adult life, and affects regions including the cortex, hippocampus, and striatum (Mantamadiotis et al., 2002). Together, these two experiments suggest that while, individually, CREB and CREM are dispensable for the general establishment and early formation of the CNS, the presence of at least one of these factors is essential for neuronal survival at late embryonic and postnatal ages. Further, the relatively mild CNS phenotypes of either single gene mutant compared to the dramatic deficits observed in the double mutants implies that the upregulation of *Crem* that is reported in brains of both hypomorphic *Creb* mutants (Hummler et al., 1994) and *Creb* conditional mutants (Mantamadiotis et al., 2002) is functionally important in vivo. Thus other family members appear

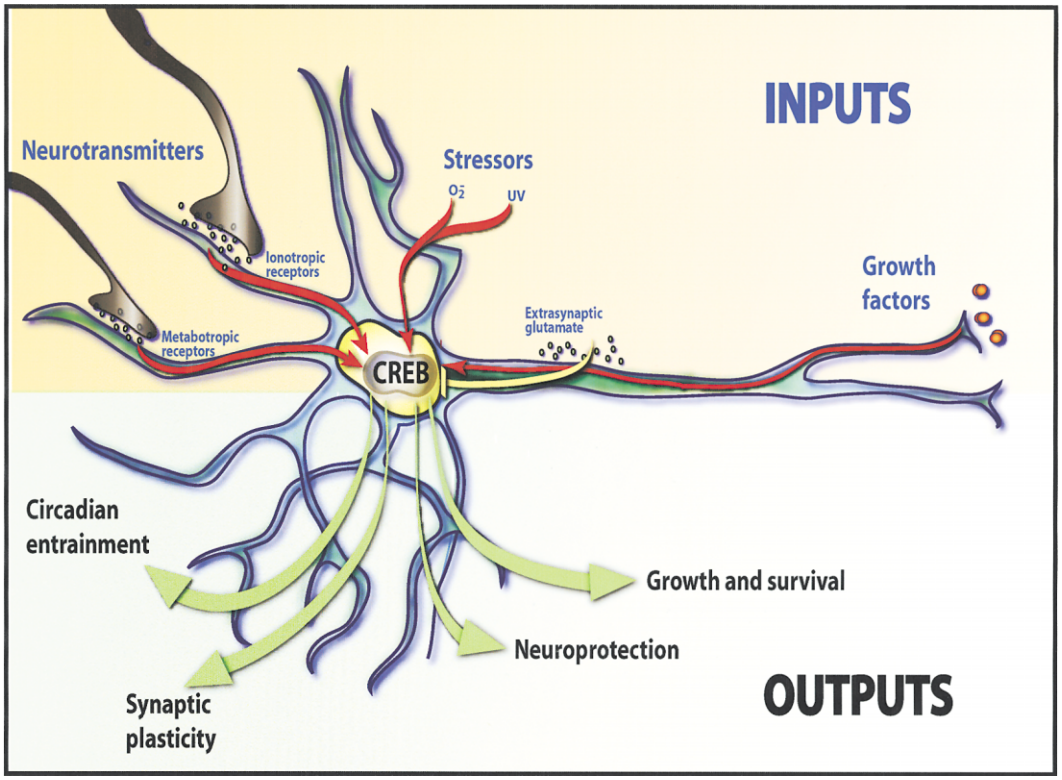

Figure 2. CREB-Dependent Gene Expression Is Critical for a Variety of Functions in the Developing and Mature Nervous System

Shown are some of the processes for which CREB-dependent gene expression has been implicated. Stimuli and conditions that promote CREB phosphorylation and CREB-mediated gene expression are indicated in the upper half of the diagram, while physiological and pathological consequences of CREB activation are depicted in the lower half.

to play, at minimum, compensatory roles in vivo in the absence of CREB, but likely also play complementary roles in vivo in its presence. The latter possibility is strongly supported by the finding that *Crem* null mice exhibit excess apoptosis in germ cells (Nantel et al., 1996). Moreover, mouse embryos lacking both CREB and ATF-1 die prior to implantation, and embryos lacking CREB but retaining a single functional *Atf-1* allele die at E9.5 (Bleckmann et al., 2002). This early embryonic lethality is attributed to widespread apoptosis of multiple cell types.

CREB, Proliferation, Neuronal Differentiation, and Process Outgrowth. The extent to which CREB family members are required in the nervous system for developmental processes other than survival has only begun to be explored. Both in vivo and in vitro studies of sensory neurons derived from *Creb* null mice have revealed that axonal growth is compromised in the absence of CREB, and that these growth defects occur independently of neuronal survival defects (Lonze et al., 2002). Moreover, *Creb* null mice display defects in axonal projections within at least two major brain commissures (Rudolph et al., 1998), and in vitro, dominant-negative inhibitors of CREB family members attenuate outgrowth of cortical neuron dendrites (Redmond et al., 2002). In addition to neurite outgrowth, it is plausible that CREB also supports developmental processes such as precursor proliferation. There is precedent for CREB functioning as a factor critical for proliferation of non-neuronal cell types such as pituitary somatotrophs and chondrocytes (Long et al., 2001; Struthers et al., 1991), and further, CREB activation has been found to correlate with axon-induced alterations in proliferation of Schwann cells during development of the peripheral nervous system (Lee et al., 1999; Stevens and Fields, 2000). The characterization of neuronal precursor proliferation in *Creb* null embryos, however, is as of yet not comprehensive. While DRG precursors appear to proliferate normally in the absence of CREB, the finding that SCGs are never normally populated in *Creb* null embryos is consistent with the possibility that their precursors may in fact depend upon CREB for normal proliferation (Lonze et al., 2002).

CREB in Learning, Memory, and Plasticity

Early behavioral studies of learning and long-term memory revealed, through the use of pharmacological inhibitors, a requirement for both new protein synthesis and new gene transcription in these processes, and subsequent studies have demonstrated that this requirement applies also to long-term changes in synaptic plasticity, the cellular correlate of memory (reviewed in Kandel, 2001; Silva et al., 1998). The identification of specific molecules involved in learning, memory, and plasticity and the development of means by which to manipulate them represented a major advance in our understanding of these phenomena. A series of breakthrough studies were carried out using the mollusk *Aplysia*, which exhibits a memory-like behavior known as sensitization that

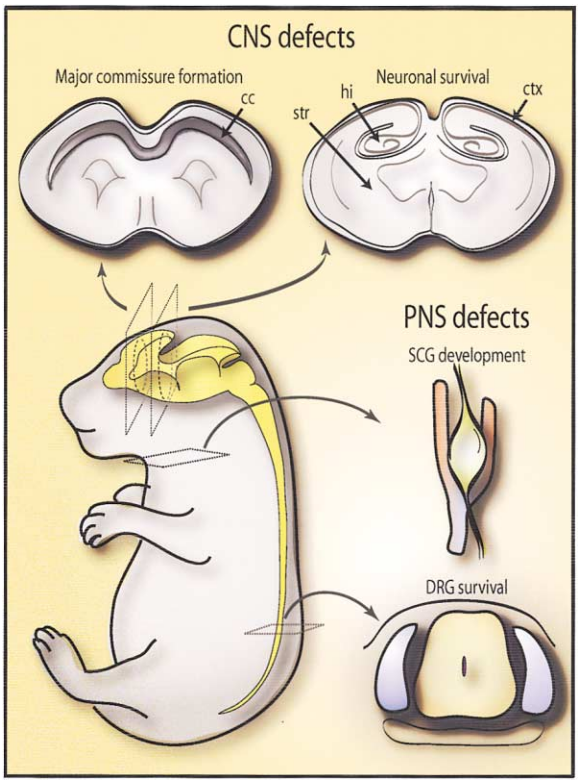

Figure 3. CREB and CREM Are Required for Proper Development of the Nervous System

In *Creb* null mice, growth defects are observed in the corpus collosum (upper left), the anterior commissure, spinal and cranial nerves, and sympathetic ganglia (middle right). Moreover, excess apoptosis is observed in sensory neurons of the dorsal root (lower right) and trigeminal ganglia. In mice lacking both *Creb* and *Crem* during development of the nervous system, massive loss of neurons is detected in a variety of brain regions, including the cortex, hippocampus, and striatum (upper left and right). CC, corpus collosum; ctx, cortex; hi, hippocampus; str, striatum.

can be conveniently recapitulated in culture in a paradigm known as long-term facilitation (LTF; reviewed in Kandel, 2001; Montarolo et al., 1986). This system provided the first mechanistic insights into the role of activity-dependent gene expression in learning and memory by demonstrating a requirement for the cAMP-CREB pathway (Dash et al., 1990; reviewed in Kandel, 2001), and paved the way for a series of elegant studies demonstrating both necessity and sufficiency of the CREB transcriptional pathway for LTF (Alberini et al., 1994; Bartsch et al., 1995, 1998; Kaang et al., 1993; reviewed in Kandel, 2001).

In addition to *Aplysia*, the fruitfly, *Drosophila melanogaster*, has also been exploited for similar studies. The development of the olfactory memory paradigm as a simple behavioral assay for learning and memory in *Drosophila* enabled geneticists to screen for mutant genes that disrupted these processes (reviewed in Waddell and Quinn, 2001). Remarkably, these genes turned out to include key regulators of intracellular cAMP levels. In particular, two genes, *Rutabaga* and *Dunce*, which when disrupted were known to impart profound learning and memory deficits, were later found to encode, respectively, a Ca^{2+}/CaM-sensitive adenylate cyclase and a phosphodiesterase (reviewed in Waddell and Quinn, 2001). CREB itself was first implicated in learning and memory in flies by experiments in which inducible expression of a CREB activator was found to enhance and inducible expression of a CREB repressor was found to block the formation of long-term memory in the olfactory task (Yin et al., 1994, 1995).

These and other studies in *Aplysia* and *Drosophila* implied that CREB plays a role in learning and memory in invertebrates, and much subsequent effort has been devoted to determining whether the same holds true for vertebrates. Among the first studies in rodents that supported a role for CREB in learning, memory, and synaptic plasticity were those in which manipulations at multiple steps of the cAMP-PKA pathway were found to alter these processes. First, mice with a targeted mutation in the type I adenylate cyclase were found to have defects both in spatial memory and in L-LTP in the CA1 region of the hippocampus (Wu et al., 1995). Further, in slice cultures, cAMP analogs (Huang et al., 1994) and antagonists (Frey et al., 1993) were found to have opposing effects on hippocampal LTP; the former triggers LTP in the absence of a tetanizing stimulus, and the latter blocks L-LTP. Finally, in mice in which activity of the cAMP effector PKA was blocked by means of a transgene expressing a PKA inhibitor, both spatial learning and L-LTP were impaired (Abel et al., 1997).

Given that multiple components of the cAMP-PKA pathway are involved in learning and memory, it was reasonable to suspect that CREB-dependent gene expression would be activated in the process as well. Indeed, robust CREB phosphorylation and CRE-reporter gene expression can be detected in cortical neurons during developmental plasticity (Pham et al., 1999) and in hippocampal neurons in response to both LTP-inducing stimuli and memory training tasks (Davis et al., 2000; Deisseroth et al., 1996; Impey et al., 1998b, 1996; Matthies et al., 1997; Schulz et al., 1999; Stanciu et al., 2001; Taubenfeld et al., 2001; Viola et al., 2000). CREB-dependent gene expression appears to be required for rather than a consequence of learning and memory because intra-hippocampal infusion of CREB antisense oligos produces deficits in spatial learning in rats (Guzowski and McGaugh, 1997). In a related study, CREB signaling was reported to be necessary for plasticity in an in vitro model of cerebellar LTD (Ahn et al., 1999). These findings support a model in which CREB-dependent gene expression contributes critically to long-term memory and plasticity in vertebrates, and they have provided the impetus to attempt the direct manipulation of CREB levels in vivo. Curiously, however, such genetic experiments have ultimately produced an array of reports that embody great complexity, and in some circumstances outright contradiction.

The first mouse genetic tool that lent itself to behavioral studies harbors a hypomorphic mutation in the *Creb* gene (Hummler et al., 1994). While this *Creb* hypomorph lacks expression of the predominant forms of CREB, α and Δ, these "α/Δ mutant" mice are able to produce trace amounts of $CREB_{\beta}$, which can carry out, at least to a modest extent, some CREB-mediated functions in vivo (Blendy et al., 1996). The first such study using these mice reported deficits in both spatial mem-

ory, and in hippocampal LTP, suggesting that in mice as in invertebrates, CREB may be required for long-term memory (Bourtchuladze et al., 1994). Results of more recent studies, however, imply either that CREB is dispensable for certain forms of hippocampal plasticity, or that the loss of CREB per se may be overcome by means of compensation provided by other family members in vivo (Gass et al., 1998; Kogan et al., 1997; Pittenger et al., 2002).

Alternative strategies for inhibiting CREB in mice have largely supported the notion that CREB is involved in certain forms of memory, but its requirement for plasticity is not absolute. Silva and coworkers recently reported that inducible activation of a CREB inhibitor blocks consolidation of long-term fear-conditioned fear memories (Kida et al., 2002). Moreover, Kandel and colleagues recently found that inducible expression of a dominant inhibitor of all CREB family members in the dorsal hippocampus produces spatial memory deficits (Pittenger et al., 2002). Interestingly, the latter study also revealed a differential requirement for CREB family members for the expression of different types of LTP. While the CREB family inhibitor had no effect on L-LTP induced either by theta bursts or tetanic trains, it did attenuate L-LTP induced by cAMP, and by the pairing of electrical stimulation with neurotransmitter application (Pittenger et al., 2002).

In addition to loss-of-function studies designed to discern the necessity for CREB in learning, memory, and plasticity in vertebrates, gain-of-function strategies have been developed to address the sufficiency of CREB in these processes. In considering a mechanism by which a transcription factor such as CREB might be involved in these processes, one question ultimately arises that is central to the understanding of synaptic plasticity: how is synapse specificity achieved? If CREB and its family members are both necessary and sufficient for synaptic modifications, how could activity at one synaptic site activate CREB in the nucleus such that its plasticity-supporting outputs could be restricted to the activated synapse?

Perhaps the most provocative model is the "synaptic tagging" or "synaptic capture" model, in which plasticity-inducing stimuli result in the "activation" of the postsynaptic neuron, and the nuclear outputs of this activation are captured by tags at active synaptic sites, including those that receive weaker inputs. Support for this model derives from studies of the synapse specificity of LTF in invertebrate sensory neurons (Casadio et al., 1999; Martin et al., 1997) and of LTP in vertebrate hippocampal neurons (Frey and Morris, 1997). Such a model provides a mechanistic explanation for the observation that subthreshold stimulation of a given synapse can result in LTP at that synapse if LTP had previously been induced at a separate synapse (Frey and Morris, 1997). In a recent study, Kandel and colleagues have provided evidence both to support the synaptic tagging model and to suggest a means by which CREB-dependent gene expression may be involved (Barco et al., 2002). They propose that a central activating event that follows the LTP-inducing stimulus is the activation of CREB; this results in the cell-wide distribution of CREB-dependent gene products which can be "captured" by

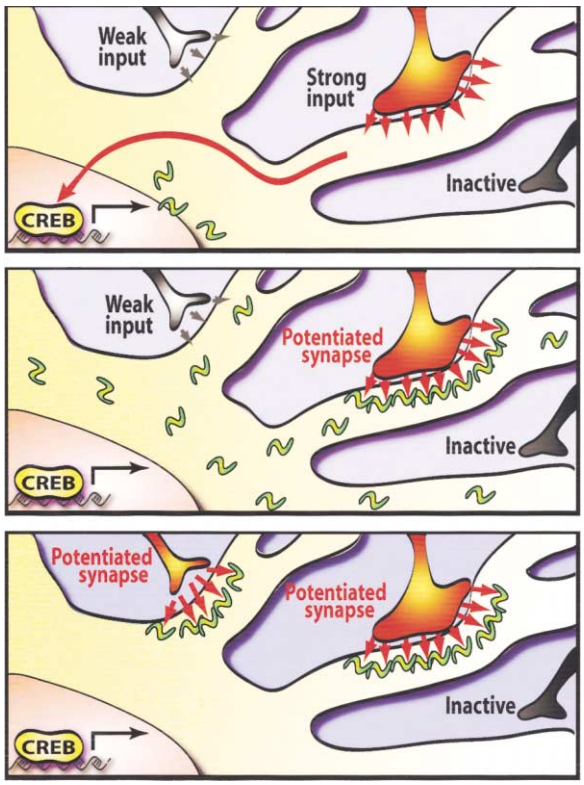

Figure 4. The Products of CREB Genes Are Captured to Potentiate Synapses during Synaptic Plasticity

An interesting problem in the development of models to explain the role of activity-dependent gene expression in long-term changes in synaptic efficacy is how input specificity is achieved. The synaptic tagging or synaptic capture model posits that, while activity-dependent gene products are distributed throughout the neuron, they are captured by "tags" present only at sites of synaptic stimulation. Current work suggests that these activity-dependent gene products are likely to include CREB target genes. As depicted, strong synaptic inputs are required for activating CREB in the nucleus (upper panel). The products of CREB-dependent gene expression are distributed throughout the neuron and may be captured by any active synapse (middle panel), such that even a weak synapse may be specifically potentiated (lower panel). Inactive synapses are incapable of capturing the nuclear gene products, and therefore remain inactive (middle and lower panels).

local tags only at activated synapses (Figure 4). According to such a model, in an "activated" neuron, even a subthreshold stimulus would suffice to potentiate a synapse, and this is indeed what is observed when a constitutively active form of CREB is inducibly expressed in hippocampal neurons in vivo (Barco et al., 2002). Thus while CREB alone may not be absolutely required for the expression of LTP, the products of its target genes appear to provide sufficient signals for synaptic strengthening. The identities of these CREB target genes, and the mechanisms by which their protein products are captured, remain questions to be addressed in future studies.

CREB and Addiction

Recently, much effort has been devoted to unraveling the genetic underpinnings of addiction. While this seems a daunting task, considering the incredible complexity of this phenomenon, the problem may be greatly simpli-

fied if approached from the standpoint of studying stimulus-induced, long-lasting changes in neuronal function, much as has been the approach to studying learning and memory. In this light, it may not be surprising to find that addiction, like learning and memory, is a process in which activation of new gene transcription plays an important role, and, consequently, is a process in which CREB and CREB-dependent gene expression have been implicated.

Several lines of evidence have pointed to a role for CREB in the long-term neuronal changes associated with addiction. The first studies simply entailed the characterization of CREB expression and activation in the brain in response to substances of abuse. Interestingly, in CNS regions known to be involved in addiction, such as the locus coeruleus and nucleus accumbens, CREB phosphorylation and CRE-mediated gene expression were found to vary dramatically in a manner dependent upon exposure to drugs including opiates, cocaine and amphetamines, nicotine, and ethanol (reviewed in Berke and Hyman, 2000; Blendy and Maldonado, 1998; Nestler, 2001). These observations provided strong correlative evidence that CREB-dependent gene expression is involved in both the acute responses to substances of abuse and the development of addiction. The suggestion that CREB is directly involved has been largely substantiated by in vivo gain- and loss-of-function experiments (Carlezon et al., 1998; Maldonado et al., 1996; Self et al., 1998; Walters and Blendy, 2001). Interestingly, the regulation of CREB phosphorylation and the function of CREB in addiction varies with respect to multiple parameters, including the identity of the substance (opiates versus cocaine), the nature of the exposure (acute versus chronic), and the CNS region involved. For example, while both morphine and cocaine appear to regulate CREB levels or activity in brain regions involved in reward, they appear to achieve opposing effects: chronic morphine decreases levels of total CREB protein (Widnell et al., 1996) while cocaine administration induces CREB phosphorylation (Kano et al., 1995). These opposing effects appear to be preserved at the behavioral level since $CREB^{\alpha/\Delta}$ mutant mice are insensitive to the positive reinforcing properties of morphine but are hypersensitive to those of cocaine (Walters and Blendy, 2001). Further examples and greater details need not be provided here as they have been discussed in depth elsewhere (reviewed in Blendy and Maldonado, 1998; Hyman and Malenka, 2001; Nestler, 2001); however, what emerges from this collection of work is the general observation that CREB-dependent gene expression, while it may be universally involved in addiction, does not play a universal role under all circumstances. Moreover, it seems clear that future research will be necessary to delineate the potential functions of the CREB family members, CREM and ATF1, in addiction.

CREB in Circadian Rhythms

The entrainment of the circadian clock is yet another fascinating and complex physiological process in which CREB-dependent gene expression appears to be intricately involved. The SCN of the anterior hypothalamus, the site of the endogenous circadian clock in mammals, is known to respond to light from the external world via a subset of retinal afferents that synapse directly upon SCN neurons (reviewed in Cermakian and Sassone-Corsi, 2000; Reppert and Weaver, 2001). This neuronal circuit is capable of phase-shifting the clock because the retinal afferents that synapse in the SCN activate gene expression in their postsynaptic neurons only when they encounter light during the subjective night, not during the subjective day. This temporal restriction applies to the activation of CREB and the induction of immediate early genes (IEGs) as well; CREB is phosphorylated (Ginty et al., 1993) and IEGs are transcribed (reviewed in Kornhauser et al., 1996) in SCN neurons upon exposure to light only during the subjective night. Normally, CREB target genes are expressed in a circadian time-dependent manner (Obrietan et al., 1999). These genes are believed to be instrumental in the resetting of the clock in response to light (Gau et al., 2002; reviewed in Reppert and Weaver, 2001; Travnickova-Bendova et al., 2002), and it is likely that CREB activation in the SCN serves to phase-shift the clock by making modifications in the relative levels of these cycling transcripts. In support of this model, Schütz and coworkers have recently demonstrated a requirement for CREB-dependent gene expression in the SCN in vivo for light-induced phase-shifting of the clock (Gau et al., 2002).

In addition to a role in entrainment of the clock, CREB family members have been implicated as key players that function within the pineal gland to establish and maintain the diurnal circadian cycle of melatonin synthesis. In a mechanism that involves the functions of both CREB and the CREB-inducible repressor ICER, a transcriptional regulatory loop is established in which CRE-mediated gene expression cycles between periods of activity and inactivity (Foulkes et al., 1997; Stehle et al., 1993). The consequence is the rhythmic expression of CREB target genes, such as the rate-limiting enzyme in melatonin synthesis that, in living organisms in the environment, fluctuates in synchrony with the day-night cycle.

CREB in Neuroprotection and Disease

Interestingly, CREB is activated not only in response to the pro-growth and pro-survival stimuli described above, but in response to stressful stimuli as well (Deak et al., 1998; Iordanov et al., 1997; Tan et al., 1996; Wiggin et al., 2002). In neurons, CREB is phosphorylated under conditions of hypoxia and oxidative stress, suggesting that the activation of a CREB-dependent survival program in response to harmful stimuli might represent a cellular form of defense. Indeed several pieces of evidence strongly support a role for CREB as a neuroprotectant.

In animal models, transient ischemic insults are capable both of phosphorylating CREB (Hu et al., 1999a; Mabuchi et al., 2001; Tanaka et al., 2000; Walton et al., 1999a) and inducing CRE-driven transgene expression (Mabuchi et al., 2001) in neurons. The functional importance of CREB activation in response to stress is implied by the provocative observation that, in some cell types, differential susceptibility to hypoxia-induced cell death correlates well with the ability to sustain CREB phosphorylation. In the hippocampus, temporary ischemia produces a phosphorylation of CREB that is transient in CA1 neurons but is prolonged in dentate gyrus (DG) neurons (Hu et al., 1999a; Walton et al., 1999a). Remarkably, while the CA1 neurons are dramatically depleted following the ischemic insult, DG neurons are substan-

tially spared, and there is reason to believe that the neuroprotection in the DG may directly involve CREB-dependent processes. This neuroprotection appears to require CREB-dependent gene expression because injection of CRE decoy oligos exacerbates cell death following excitotoxic stimuli (Mabuchi et al., 2001). Furthermore, in PC12 cells, hypoxia induces the expression of the CREB-dependent pro-survival gene *bcl-2* (Freeland et al., 2001), and in cultured neurons, *bcl-2* is upregulated in cells that ultimately withstand harmful stimuli (Mabuchi et al., 2001). These observations suggest a model by which cellular insults are capable of initiating two parallel signals, one that encodes the nature of the injury and a second that, perhaps by default, activates a CREB-directed pro-survival program; thus the fate of a cell could be determined by the relative strengths of these two signals.

Because CREB is central not only to cell survival but also to many other physiological processes, it is not surprising that the consequences of disrupting CREB function in vivo are quite severe. While the complete disruption of CREB is lethal in mice (Rudolph et al., 1998) and is therefore not likely to be compatible with human life, several pathological conditions exist in which CREB function may be disrupted subtly or incompletely. Some such conditions are genetic disorders in which CREB interactors are defective. The gene encoding RSK-2, one of several putative CREB kinases, for instance, is mutated in Coffin-Lowry syndrome, a complex disorder characterized by multiple physical abnormalities and mental retardation (Trivier et al., 1996). In addition, a heterozygous mutation in the *CBP* gene produces Rubenstein-Taybi Syndrome, which is similarly characterized by multiple deficits including mental retardation (Petrij et al., 1995).

In most cases, the mechanism by which the disruption of CREB function may produce disease is poorly understood. One notable exception is the group of polyglutamine repeat diseases, which includes Huntington's Disease (HD), a devastating neurodegenerative disorder. The molecular events that contribute to these diseases have been the focus of intense investigation, and recently the CREB interactors CBP, P/CAF, and $TAF_{II}130$, have been implicated as central to the pathophysiology of HD (reviewed in Dawson and Ginty, 2002). Specifically, several independent groups have found that mutant huntingtin protein (the causative agent in some forms of HD), via its expanded polyglutamine tract, forms nuclear aggregates that contain CBP (McCampbell et al., 2000; Nucifora et al., 2001; Steffan et al., 2000). This sequestration of CBP presumably compromises transcription of CBP-dependent genes, and may contribute directly to the neuronal death associated with HD. Among the most provocative pieces of evidence in support of this is the finding that the cell death accompanying expression of mutant huntingtin can be partially rescued by overexpression of CBP (Nucifora et al., 2001). Thus the ability of CBP to interact with its various transcription factors, including CREB, appears to be required for neuronal survival in some circumstances. Interestingly, the survival-promoting effects of CBP may depend also upon its activity as a histone acetyltransferase (HAT), through which it is believed to modify local chromatin and enable the basal transcription machinery to access the promoter (Bannister and Kouzarides, 1996). In support of this possibility, recent work has shown that histone deacetylase inhibitors can reduce cell death associated with the expression of polyglutamine expansion proteins (McCampbell et al., 2001; Steffan et al., 2001).

Together these findings suggest that disrupting CBP function is at least one mechanism central to the pathology of polyglutamine repeat diseases. But since CBP is a transcriptional coactivator not specific to CREB alone, the extent to which dysfunction of CREB family members contributes to neurodegenerative diseases is not clear. It is interesting to note that at least one recent study in which microarray analyses were used to compare transcriptional profiles of cells expressing either wild-type or mutant huntingtin turned up a number of genes that contain CRE sequences in their promoters (Wyttenbach et al., 2001). In addition, other approaches that complement gene expression analyses have proven equally useful in elucidating the potential role of CREB in these disorders. Indeed, recent mouse genetic studies by Schütz and colleagues have shown that the ablation of both *Creb* and *Crem* in the postnatal CNS produces a progressive and profound neurodegeneration that is widespread but not universal throughout the CNS (Mantamadiotis et al., 2002). This phenotype is of keen interest because such a pattern of neuronal loss closely parallels that which is observed in several clinical neurodegenerative disorders, including HD. Such sophisticated genetic models will likely serve as powerful tools that can be used to understand the means by which CREB supports neuronal maintenance, and to delineate the role of CREB family members in the many diseases in which they are now only casually implicated.

CREB Target Genes

The discovery that CREB in neurons is critically important for growth, survival, and synaptic function has naturally prompted the question of how, mechanistically, CREB acts under these and other circumstances. Intuitively, it is easy to understand that each of these different processes depends upon the proper activation of a specific program of gene expression. Given that each process requires CREB family members, is it then also the case that each depends upon the activation of a specific program of CREB-dependent gene expression? The answer to this question has begun to be revealed by the identification and expression patterns of CREB target genes.

Specificity in CREB-Dependent Gene Expression

The list of putative CREB target genes now exceeds 100, and includes genes that control neurotransmission, cell structure, signal transduction, transcription, and metabolism (Table 1; also reviewed in Mayr and Montminy, 2001). By definition, all CREB target genes share in common the presence of one or more CREB binding sites in their promoter regions, which confer some degree of CREB-mediated regulation. But beyond this defining characteristic, many CREB target genes share few other similarities, and perhaps this is to be expected given the wide variety of possible changes that can be brought about when CREB is activated under different circumstances. This functional diversity of CREB target

Table 1. An Abbreviated List of Genes with CRE Sequences

Neurotransmission/Peptides	Acetylcholinesterase (AchE), α1-GABAA receptor, β1-adrenergic receptor, β2-adrenergic receptor, Cardiotrophin-1 (CT-1), Calcitonin gene-related peptide (CGRP), Cholecystokinin (CCK), Chromogranin A, Chromogranin B, Corticotropin-releasing hormone, Dopamine β-hydroxylase (DβH), Enkephalin, Galanin, Galanin receptor1 (GalR1), Glycoprotein hormone α subunit, Gonadotropin-releasing hormone receptor (GnRHR), Human chorionic gonadotropin-α (hCG-α), Murine gastrin-releasing peptide receptor (mGRP-R), Neurotensin/neuromedin N (NT/N), Norepinephrine transporter (NET), Pituitary adenylyl cyclase activating polypeptide (PACAP), Preprotachykinin A, Prodynorphin, Proenkephalin, Proglucagon, Secretogranin, Secretogranin II, Somatostatin, Somatostatin receptor (ssr-2), Substance P receptor, Synapsin I, Tyrosine aminotransferase, Vasoactive intestinal polypeptide (VIP), Vasopressin (AVP), Vesicular monoamine transporter (VMAT), VGF
Growth Factors/Hormones	Brain derived neurotrophic factor (BDNF), Fibroblast growth factor-6 (FGF-6), Flt-1, Insulin-like growth factor I (IGF-I), Inhibin α, Leptin, Transforming growth factor-β2 (TGF-β2), TrkB
Structural	E-cadherin, Fibronectin, ICAM-1, Neurofilament 68 kDa (NF-L)
Channels/Transporters	Aquaporin-2 (AQP-2), Cystic fibrosis transmembrane conductance regulator (CFTR), Glucose transporter 2 (GLUT2), Kv3.1 K^+-channel, Na^+/K^+-ATPase α
Cellular Metabolism	Arylalkylamine N-acetyltransferase (AA-NAT), Bcl-2, Cyclooxygenase-2 (COX-2), Cytochrome c, Glutamine synthetase (GS), Heme oxygenase-1 (HO-1), Hexokinase 2, HMG-CoA synthase, Neuron specific enolase (NSE), Ornithine decarboxylase, Phosphoenolpyruvate carboxykinase (PEPCK), Pyruvate carboxylase, Superoxide dismutase 2 (SOD2), Ubiquitin-conjugating enzyme, Uncoupling protein-1 (UCP1), Uncoupling protein-2 (UCP2), Uncoupling protein-3 (UCP3)
Transcription	Activating transcription factor-3 (ATF-3), C/EBP-β, c-fos, CREB, Egr-1, ICER, JunD, Krox-20, mPer1, mPer2, Nurr1, Pit-1, STAT3
Signal Transduction	14-3-3-ϵ, Cyclin A, Cyclin D1, Inducible nitric oxide synthase (iNOS), Neurofibromatosis 1 (NF-1), Neuronal nitric oxide synthase (nNOS), Prostaglandin synthase-2 (PGS2), Serum and glucocorticoid inducible kinase

genes may have intriguing mechanistic implications. Namely, while all CREB target genes contain CRE sequences, it is unlikely to be the case that this CRE sequence functions as a simple switch by which the entire portfolio of CRE-driven genes is coordinately turned on when CREB is phosphorylated, and turned off when CREB is dephosphorylated. Indeed the expression patterns of a few well-characterized genes serve to argue strongly against this possibility.

Perhaps the most familiar of such examples is that of genes whose expression kinetics are tightly temporally regulated. These include stimulus-inducible genes that can be categorized into groups of immediate early genes (IEGs), intermediate, and delayed response genes, based on the time after stimulation at which they are maximally transcribed. c-*fos* is the best-characterized example of an IEG, whose transcription in multiple cell types is rapidly but transiently activated upon a wide variety of stimuli (Sheng and Greenberg, 1990). In a landmark discovery, Greenberg and Ziff found that c-*fos* transcription peaks within minutes of stimulation, but invariably returns to baseline within 1 hr of the stimulus onset, independent of the duration of the stimulus (Greenberg and Ziff, 1984). Interestingly, this prototypic IEG was one of the first identified CREB target genes, whose expression is induced in a CRE-dependent manner in response to certain stimuli that activate CREB (Berkowitz et al., 1989; Fisch et al., 1989; Ginty et al., 1994; Sassone-Corsi et al., 1988; Sheng et al., 1990). But while CREB may be required to initiate c-*fos* expression, its transcriptional activity does not precisely correlate with CREB phosphorylation because even in the presence of stimuli that produce prolonged phosphorylation of CREB, c-*fos* transcription is still transitory.

Thus, kinetic differences in expression profiles demonstrate that CREB functions by a mechanism more complex than a simple switch. This point is further underscored by the observation that stimuli that activate CREB can bring about different consequences depending upon the cell type on which they act. For example, the CREB target gene *Bdnf* (Shieh et al., 1998; Tao et al., 1998) is robustly induced in hippocampal neurons upon synaptic stimulation (Patterson et al., 1992; Zafra et al., 1990), but in some other types of neurons, *Bdnf* is not expressed. This tissue specificity of *Bdnf* expression occurs despite the fact that CREB activation is required for *Bdnf* expression in hippocampal neurons, and that CREB activation actually occurs in most, if not all types of neurons in response to activity. Conversely, the *tyrosine hydroxylase (TH)* gene is a CREB-dependent gene (Cambi et al., 1989) that is highly expressed in sympathetic neurons but not in hippocampal neurons in response to growth factor stimulation, even though growth factors lead to CREB phosphorylation in both cell types. What accounts for the specific ability of CREB to drive expression of *Bdnf* and *TH* in distinct neuronal populations is as of yet unclear, but presumably reflects its ability to function in an integrative manner with cell type-specific transcription factors and coactivators (for example, see Tao et al., 2002).

A final example of the striking specificity of CREB-dependent gene expression is the observation that within a given cell, CREB phosphorylation occurs in response to many types of stimuli. It is clear that a stimulus that induces, for instance, cell survival must activate a set of target genes distinct from the set of target genes activated by a stimulus that induces proliferation. And indeed different stimuli are capable of activating differ-

ent subsets of CREB target genes. For example, in PC12 cells, stimuli that activate either PKC or PKA are capable of producing comparable levels of phosphorylated CREB (Mayr et al., 2001; Thompson et al., 1995), yet only stimuli that activate PKA are capable of inducing transcription of the CREB target gene *ICER* (Mayr et al., 2001). The mechanisms by which this stimulus specificity is achieved are not known.

Reports in the literature of genes whose regulatory regions contain CRE or CRE-like sequences now number well into the hundreds. Despite this, however, the extent to which expression of these genes truly depends upon CREB, CREM, or ATF-1 is unknown in the vast majority of cases. Thus, while the involvement of CREB family members in many various processes is now well established, the identities of their direct target genes remain a predominating question. Rapidly evolving technologies for high-throughput gene expression analyses will undoubtedly enable a comprehensive characterization of these genes, and such information could prove invaluable in deciphering the mechanisms by which this factor can bring about context-specific change. For instance, the identification of coordinately regulated subsets of CREB target genes could reveal that certain subsets of CREB target genes are activated only when CREB cooperates with other transcription factors. Indeed *c-fos* is an example of one such gene whose growth factor-induced expression requires the cooperation of activated CREB and SRF (Bonni et al., 1995). Such information could very well lead to the discovery of other regulatory mechanisms that must exist to account for the remarkable specificity of CREB-dependent gene expression.

CREB Signaling and Regulation

Since the identification and cloning of CREB, the molecular mechanisms by which it functions as an inducible regulator of transcription have been the focus of much investigation. This work has described many of the signaling events that convert extracellular stimuli into CREB activation, and has identified Ser-133, within the KID domain, as a key regulatory site which must be phosphorylated in order for CREB to function as a stimulus-dependent transcriptional activator. In neurons, CREB phosphorylation occurs under a wide variety of cellular circumstances, which share in common only that each is initiated by external stimuli. These include responses to growth factors during the development of the nervous system, depolarization and synaptic activity during normal neuronal function, and hypoxia and stress responses during stroke or neural injury. Thus CREB is thought to play the role of a general stimulus-inducible factor, and this characteristic implicates it as a key participant in one biological phenomenon whose mechanism has largely remained an unanswered question. Namely, how do diverse inputs, which produce an equally diverse array of outputs, all function by means of a common effector? In this section, we will consider the pathways that connect external stimuli to CREB-activated gene expression (Figure 5), and begin to explore the extent to which CREB-dependent transcription is responsible for mediating the differential effects of these stimuli.

Signaling Pathways and the Kinases that Lead to Phosphorylation of CREB

cAMP signaling to CREB. An investigation of the mechanisms of cAMP-induced somatostatin transcription led Goodman, Montminy, and colleagues to seminal discovery of the cAMP response element (CRE) within its promoter (Montminy et al., 1986). This represented a major advance in the understanding of stimulus-regulated gene expression and ultimately led to the cloning of the CRE binding protein, CREB (Gonzalez et al., 1989; Hoeffler et al., 1988). Subsequently, the link between cAMP and CREB-dependent gene expression was found upon the important discovery of CREB Ser-133 phosphorylation as a modification central to CREB activation (Gonzalez and Montminy, 1989). The kinase responsible for this activating phosphorylation event was identified as the cAMP-dependent protein kinase, PKA (Gonzalez and Montminy, 1989). PKA activity is regulated by molecules that alter cAMP levels, and therefore by molecules that regulate adenylate cyclase activity, perhaps the best characterized of which are the G protein-coupled receptors (GPCRs). In the nervous system, GPCRs function as receptors for functionally important ligands including many neurotransmitters. By means of these receptors, neurotransmitters and neuropeptides can couple to cAMP, PKA, and, ultimately, CREB. The ability to activate CREB via PKA, however, is not restricted to ligand activation of GPCRs because some subtypes of adenylate cyclases are regulated by Ca^{2+}. Thus CREB activation via PKA can also occur in response to the many stimuli capable of increasing intracellular Ca^{2+} (reviewed in Poser and Storm, 2001).

Ca^{2+} Signaling to CREB. Early work from Greenberg and others demonstrated that multiple neurotransmitters are capable of activating expression of immediate early genes in a Ca^{2+}-dependent manner (for examples, see Bading et al., 1993; Bartel et al., 1989; Greenberg et al., 1986; Morgan et al., 1987; Morgan and Curran, 1986; Sheng et al., 1990). Subsequent studies, both from the Greenberg laboratory and from Kandel and coworkers, led to the finding that CREB can function as a Ca^{2+}-inducible transcription factor (Dash et al., 1991; Sheng et al., 1991; reviewed in Shaywitz and Greenberg, 1999). In neurons, increases in intracellular Ca^{2+} are largely brought about through voltage- or ligand-gated cation channels. Upon membrane depolarization, for instance, Ca^{2+} influx occurs via voltage-sensitive calcium channels (VSCC) such as the L type Ca^{2+} channel. On the other hand, during glutamatergic synaptic transmission, for example, glutamate binds to ionotropic receptors, such as the NMDA subtype, which upon activation can function as cation-permeable ion channels. Ionic Ca^{2+} interacts with a large number of intracellular molecules, one of the most thoroughly characterized of which is the Ca^{2+} binding protein calmodulin (CaM). There is currently considerable debate regarding whether the activation of CaM necessary for signaling to the CREB kinase requires cytoplasmic (Deisseroth et al., 1998) or nuclear Ca^{2+} (Hardingham et al., 2001). Regardless of its location, Ca^{2+}-CaM can activate CaMKI, CaMKII, and CaMKIV, each of which have the capacity to phosphorylate CREB, at least in vitro (Dash et al., 1991; Sheng et al., 1991; West et al., 2001). Of these, CaMKIV has emerged as the most important Ca^{2+}-activated CREB

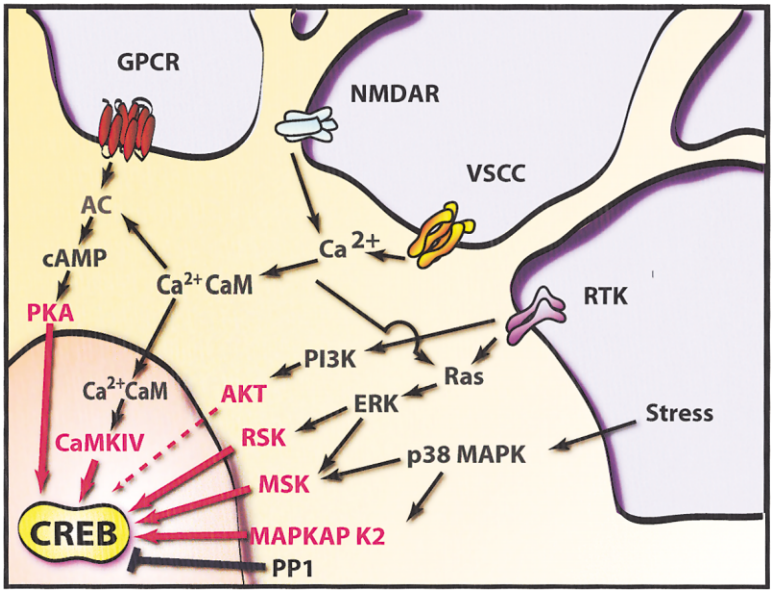

Figure 5. An Overview of Signaling Pathways that Converge on CREB

Excitatory neurotransmitters, ligands for GPCRs, neuronal growth factors, and stress inducers are among the stimuli that activate signaling pathways that converge upon CREB. As described in the text, multiple stimulus-dependent protein kinases have been implicated as CREB kinases in neurons, and a high degree of crosstalk exists between these signaling pathways. Stimulus-dependent CREB kinases include PKA, CaMKIV, MAPKAP K2, and members of the pp90RSK (RSK) and MSK families of protein kinases. Protein phosphatase 1 (PP1) has been implicated as the predominant phospho-CREB phosphatase.

kinase in vivo (Anderson and Means, 2002; Ho et al., 2000; Kang et al., 2001; Ribar et al., 2000; Wei et al., 2002). In addition to its ability to activate the CaMKs, Ca^{2+} appears to activate an additional pathway, the Ras/ERK pathway (Bading and Greenberg, 1991; Davis et al., 2000; Dolmetsch et al., 2001; Impey et al., 1998a; Roberson et al., 1999; Rosen et al., 1994; reviewed in Sweatt, 2001), which signals to an independent set of CREB kinases that are discussed below.

Growth Factor Signaling to CREB. The above examples illustrate the means by which a single stimulus, such as glutamate, can activate more than one CREB kinase by triggering parallel signaling pathways. Like glutamate, neuronal growth factors appear to have the capacity to trigger parallel pathways leading to CREB phosphorylation through the activation of a single receptor (Bonni et al., 1995; Finkbeiner et al., 1997; Ginty et al., 1994). Many such growth factors, including neurotrophins, signal via receptor tyrosine kinases which, upon ligand binding and dimerization, activate several known signaling cascades (reviewed in Patapoutian and Reichardt, 2001; Sofroniew et al., 2001). One of the best characterized is the Ras/ERK pathway, a cascade of kinases which, once triggered, ultimately results in the phosphorylation of CREB. The Ras/ERK-dependent phosphorylation of CREB can be carried out by several different kinases, among which are members of the RSK and MSK families. RSK1, RSK2, and RSK3 are each MAPK activated, and each has been shown to phosphorylate CREB in cell lines in response to growth factors (De Cesare et al., 1998; Xing et al., 1996, 1998). The structurally related protein kinases MSK1 and MSK2 are also activated by MAPK pathways (Deak et al., 1998), and their roles in growth factor-dependent phosphorylation of CREB have recently been addressed genetically. In mouse ES cells lacking MSK1 (Arthur and Cohen, 2000) and in fibroblasts lacking both MSK1 and MSK2 (Wiggin et al., 2002), CREB phosphorylation in response to stimulation with phorbol esters and growth factors such as EGF is greatly attenuated. Thus, depending on the cell type and the nature of the growth factor stimulus, one or more of these five related CREB kinases, RSK1-3 and MSK1/2, may catalyze phosphorylation of CREB Ser-133.

In addition to the MAPK pathways, receptor tyrosine kinases activate a second major signaling pathway, the PI3-kinase/Akt pathway (reviewed in Cantley, 2002). Like the MAPK pathways, for which routes to CREB are well documented, there is now evidence to indicate that, at least under some circumstances, the PI3-kinase/Akt pathway is important for CREB activation. Indeed, Akt can mediate CREB activation and CRE-mediated transcription in cell lines in response to serum or IGF-1 stimulation (Du and Montminy, 1998; Pugazhenthi et al., 2000). Moreover, recent pharmocological studies have implicated the PI3-kinase pathway in the control of CREB phosphorylation in neurons (Lin et al., 2001; Perkinton et al., 2002). However, whether Akt can directly phosphorylate CREB is not known, and it seems likely that one of the CREB kinases mentioned above catalyzes PI3-kinase and Akt-dependent phosphorylation of CREB. Nevertheless, the available evidence suggests that at least two major receptor tyrosine kinase-activated pathways may contribute to CREB phosphorylation via multiple CREB kinases. Interestingly, neurons, due to their size and morphological complexity, face a unique challenge in converting extracellular stimuli, such as neurotrophins, into nuclear signals including CREB phosphorylation. For peripheral neurons, long-range retrograde signaling can be achieved through a mechanism in which activated ligand-receptor complexes are physically transported from distal axons to the cell bodies (Riccio et al., 1997; Watson et al., 1999, 2001).

Stress-Induced Signaling to CREB. While CREB activation occurs in response to pro-growth and pro-survival stimuli as described above, it is also phosphorylated in response to harmful or stressful stimuli, including UV irradiation and hypoxia. The molecular components that comprise stress-induced signaling

cascades have not been as precisely defined as they have for growth factors, but nonetheless some of the key players are known. SAPK2/p38MAPK, a stress-activated kinase, for instance, has at least three downstream targets, MAPKAP K2, MSK1, and MSK2, which are CREB kinases (Deak et al., 1998; Tan et al., 1996). These appear to be critical regulators of CREB because in mouse fibroblasts lacking MSK1 and MSK2, CREB phosphorylation in response to stress is eliminated almost entirely (Wiggin et al., 2002).

Negative Regulation of CREB by Phosphatases. To date, two phosphatases, PP1 and PP2A, are thought to be capable of directly dephosphorylating CREB. Both PP1 and PP2A have been implicated in the removal of the Ser-133 phosphate added by PKA (Alberts et al., 1994b; Hagiwara et al., 1992; Wadzinski et al., 1993). PP1 is involved in the dephosphorylation of Ca^{2+}-activated CREB in vitro (Bito et al., 1996) and in vivo (Genoux et al., 2002), and is believed to underlie the transiency of CREB phosphorylation in response to brief electrical stimuli. Beyond this, whether and how phosphatase activity is controlled in a stimulus-inducible manner is relatively poorly understood. However, physiological contexts are now beginning to be identified in which their stimulus-dependent activation, and hence, the negative regulation of CREB, appears to be important. One exciting example of such negative regulation has been recently put forth by two groups, each of which have reported that different subtypes of NMDA receptors, NR2A- versus NR2B-containing receptors, impart very different regulatory effects upon CREB. This difference does not lie in their ability to activate the phosphorylation of CREB, but rather, interestingly in their differential abilities to trigger the active dephosphorylation of CREB. Bading and colleagues have suggested that extrasynaptically located NR2B-containing NMDA receptors, when activated, initiate an intracellular signaling pathway that acts as a CREB shutoff pathway (Hardingham et al., 2002). This observation is consistent with the finding of Sheng and colleagues that the duration of CREB phosphorylation upon NMDA receptor activation in hippocampal neurons correlates with developmental maturity, which may reflect developmental changes in the composition of NMDA receptor complexes (Sala et al., 2000). Both groups propose that the transient CREB phosphorylation is attributed to dephosphorylation carried out by the activation of a CREB phosphatase. In the latter case, the phosphatase is believed to be PP1, and in the former case, the identity of the phosphatase is not clear. Regardless, these findings suggest a provocative mechanism by which different stimuli and different signaling pathways can tightly control the kinetics and duration of CREB phosphorylation, and thus, they imply that differential control of phosphatase activity may represent an important regulatory parameter that could contribute to the specificity of CREB-dependent gene expression.

Presuming that this is in fact the case, several important mechanistic questions remain to be addressed. For instance, Ca^{2+}-dependent signaling pathways appear to activate CREB phosphatases, but which other pathways activated by different stimuli also share this capacity? Further, what is the nature of the regulation of CREB phosphatase activation? Is it a positive regulation, in which a resting and inactive phosphatase is turned on by an upstream stimulus, or is it a negative regulation, in which CREB phosphatases are constitutively active, and this basal activity is inhibited by stimuli that phosphorylate CREB? It is likely that the control of CREB dephosphorylation and inactivation depends upon the mode of the stimulus, and this is clearly an area for future investigation.

Insights Inferred from the Global View of Signaling to CREB. What can be gleaned from the above information is the general principle that the stimuli and multifunctional signaling cascades that can activate CREB are as vast in number as they are diverse. Despite the fact that Ser-133-phosphorylated CREB is a common intermediate, the diversity of these inputs is preserved at the level of their outputs; these stimuli generate very specific responses, each of which differ greatly from one another. From these observations, three important questions emerge. (1) Is CREB truly important for or involved in these stimulus-specific cellular changes, or is CREB merely a bystander that activates a standard set of genes under many conditions? Based on what little is known of the identity of CREB target genes, the latter does not appear to be the case. (2) Given that different stimuli can activate the transcription of different subsets of CREB target genes, is it possible that different routes to CREB phosphorylation are involved in establishing this specificity? Certain evidence suggests that the answer is yes. In cultured neurons, for instance, depolarization-induced increase in intracellular Ca^{2+} activates both the CaMK pathway and the Ras/ERK pathway, but it is the prolonged activation of the Ras/ERK pathway that ultimately supports CREB-dependent gene expression (Dolmetsch et al., 2001; Impey et al., 1998a; West et al., 2001; Wu et al., 2001). (3) If at any given moment, CREB is either phosphorylated at Ser-133 or it is not, how is all of this variation in stimulus-induced CREB-dependent gene expression possible if the final readout of each pathway is binary? The most likely, albeit speculative, answer is that this is not the case, and that additional parameters must exist by which CREB function is regulated.

But before making the conceptual leap to consider these hypothetical parameters, it is pertinent first to review the molecular events that occur in the time interval between the activation of a said CREB kinase and the actual initiation of CRE-driven gene transcription. A collection of studies over the past several years have provided a straightforward and seemingly conclusive outline of these events. More recently, however, evidence has begun to accumulate to suggest a model that is both more complex, and incomplete, and begins to address some of the aforementioned unanswered questions. We will consider the simple model first.

Initiation of CREB-Dependent Gene Expression: The Short Story

It is now abundantly clear that a large number of CREB kinases produce Ser-133-phosphorylated CREB, but exactly what happens next? A major advance in our understanding of mechanisms of CREB-dependent gene expression came about with the identification of the CREB coactivator, CBP, which was cloned by Goodman and colleagues in a search for phospho-CREB interactors (Chrivia et al., 1993). Subsequently, the CBP

paralog p300 was found to serve the same or similar CREB transactivating function (Arany et al., 1995; Lundblad et al., 1995). A series of structure/function analyses that followed enabled the molecular dissection of the CREB transcriptional complex, and have identified the domains within CREB that are instrumental in the assembly of this complex. The substitution of Ser-133 with Ala (the CREBm1 mutation) abolishes both CREB-CBP interaction and stimulus-induced CREB-dependent gene expression (Gonzalez and Montminy, 1989). On the contrary, mutations of other residues within the KID domain permit CREB-CBP interaction in the absence of stimulus. In one such mutant, termed $CREB_{DIEDML}$, the seven amino acids surrounding and including Ser-133 are replaced with a motif to which CBP can constitutively bind (Cardinaux et al., 2000). Alternatively, the same constitutive binding effect can be achieved by mutating CREB Tyr-134 to Phe, which lowers the K_m for PKA-mediated phosphorylation of Ser-133, providing a CBP binding site in the absence of stimulation (Du et al., 2000). These gain-of-function mutations that effectively mimic or enhance Ser-133 phosphorylation confer upon CREB the ability not only to bind CBP, but also to activate CRE reporter gene transcription in the absence of stimulation. Furthermore, Ser-133-phosphorylated CREB protein can similarly activate a CRE reporter when microinjected into fibroblasts (Alberts et al., 1994a). These observations have led to the general conclusion that Ser-133 phosphorylation within the KID domain is necessary and sufficient for stimulus-induced CREB-dependent transcription.

Initial structure/function analyses also identified the constitutive activation domain (Q2/CAD; Figure 1) as the CREB domain responsible for basal transcriptional activation. CAD is both necessary and sufficient for this basal transcription, as is demonstrated by the finding that GAL4-CAD drives a low level of GAL4 reporter expression that is unaffected by the presence of stimulus (Quinn, 1993; Xing and Quinn, 1994). Consistent with its ability to promote basal levels of gene expression, CAD was subsequently found to associate either directly or indirectly with several components of the basal transcriptional activation complex, including TFIIB, TAF110, and TBP (Felinski et al., 2001; Felinski and Quinn, 1999; Xing et al., 1995); thus, CAD functions independently of KID. The converse, however, may not be true, as CAD appears also to be required in order for KID to initiate stimulus-dependent gene expression (Quinn, 1993).

Taken together, these functions of KID and CAD lead to a simple and oft-described model of stimulus-induced CREB-mediated transcription: in the absence of stimulation, CREB, via its CAD domain, constitutively assembles the basal transcriptional machinery, and this results in a low level of CRE-driven transcription. Upon stimulation and phosphorylation of CREB at Ser-133, CBP is recruited to CREB, and thus to the CRE sequence in the promoter. The recruitment of this coactivator subserves at least two important functions. First, CBP has the ability to bind basal transcriptional components, and therefore it is believed to stabilize the pre-initiation complex that forms at the promoter (Kwok et al., 1994; Nakajima et al., 1997; Swope et al., 1996). And second, CBP, via its endogenous HAT activity (Bannister and Kouzarides, 1996), acetylates histones to facilitate the unraveling of chromatin and, in turn, increases the accessibility of the local chromatin to transcriptional machinery complexes. Through these two functions, CBP is thought to increase the transcriptional activity of the CREB-nucleated complex and thereby increase CRE promoter activity in a stimulus-dependent manner.

This straightforward model effectively integrates much of what is known about CREB function with much of what is known about the functions of the general transcriptional machinery. However, to accept this simple picture as a complete story leaves little room to accommodate an explanation for the stimulus, tissue type, or temporal specificity of CREB-dependent gene expression that occurs in vivo. Thus perhaps not surprisingly, considerable evidence now exists to suggest that this picture is one that is far from complete.

Initiation of CREB-Dependent Gene Expression: The Additional Layers of Complexity

One critical flaw in the above generalization is the assumption that Ser-133 phosphorylation is synonymous with CREB-dependent transcription. Several groups have reported that some extracellular stimuli capable of phosphorylating CREB on Ser-133, at least transiently, fail to induce CREB-dependent gene expression (Bito et al., 1996; Bonni et al., 1995; Liu and Graybiel, 1996; Mayr et al., 2001; Thompson et al., 1995). In PC12 cells, for instance, both depolarization and stimulation with peptide growth factors are capable of phosphorylating CREB to similar extents; however, transcription of CRE reporter genes occurs only in response to depolarization-induced intracellular Ca^{2+} increases; growth factors require additional regulatory events to support CREB-mediated transcription (Bonni et al., 1995).

At least two possible explanations exist that may account for this apparent discrepancy, neither of which is mutually exclusive. First, phospho-Ser-133 may not suffice to recruit CBP under all circumstances. Indeed, certain stimuli, such as mitogens and phorbol esters, are potent inducers of phosphorylation of CREB Ser-133, but fail to promote an association between CREB and CBP in intact fibroblasts and PC12 cells (Mayr et al., 2001; Wagner et al., 2000). Second, while the recruitment of CBP to CREB via phosphorylated Ser-133 appears to be sufficient to trigger gene expression in most cases, it may be suboptimal under some circumstances. Experiments arguing for the sufficiency of CBP include the two described above, expression of $CREB_{DIEDML}$ (Cardinaux et al., 2000) or injection of phospho-CREB protein (Alberts et al., 1994a), in which CBP should theoretically bind constitutively to CREB. Both manipulations are capable of activating a CRE reporter gene in the absence of stimulus. Recruitment of CBP to the promoter is likely to support transcription since a GAL4-CBP fusion protein can drive expression of a reporter gene containing GAL4 binding sites. Under some circumstances, however, GAL4-CBP can be a relatively weak activator of reporter gene expression. Interestingly, in response to the same stimuli known to activate CREB-dependent gene expression (e.g., cAMP analogs, glutamate, KCl, NGF), GAL4-CBP acquires an enhanced ability to drive GAL4 reporter gene expression (Chawla et al., 1998; Chrivia et al., 1993; Hardingham et al., 1999; Hu et al., 1999b; Impey et al., 2002; Liu et al., 1998). These observations suggest that CBP, after recruitment

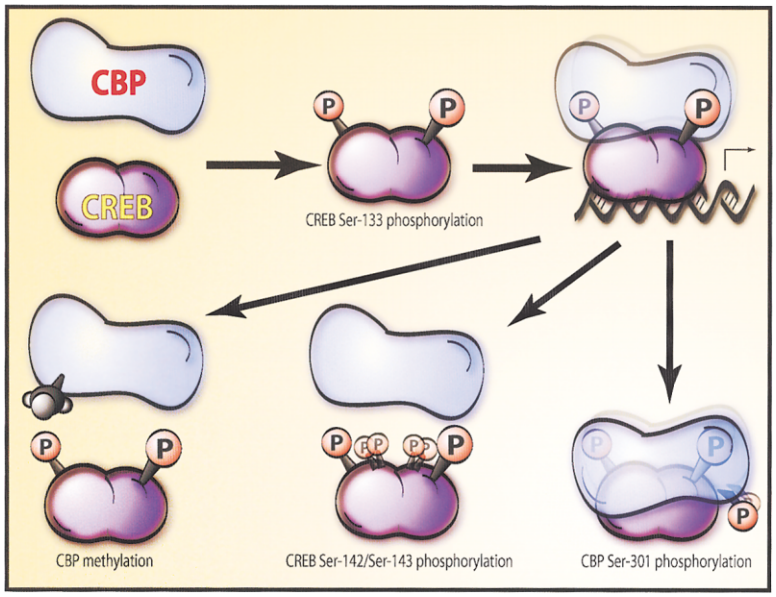

Figure 6. Phosphorylation of CREB Ser-133 and Additional Modifications of both CREB and CBP Control CREB-Dependent Gene Transcription

Phosphorylation of CREB Ser-133 serves to recruit the coactivator CBP to the promoter. CBP acetylates histones and other regulatory proteins, and interacts directly with components of the basal transcription machinery, thereby facilitating initiation of transcription. Other stimulus-dependent modifications found to affect CREB-dependent transcription and CBP recruitment include phosphorylation of CREB Ser-142 and Ser-143, phosphorylation of CBP Ser-301, and methylation of Arginine residues within the KIX domain of CBP. Interestingly, evidence indicates that both phosphorylation of CREB Ser-142/Ser-143 and methylation of CBP disrupt CREB-CBP interactions, but these modifications may not in all cases disrupt CREB-mediated gene expression. While the significance of phosphorylation of CREB Ser-133 is well understood, the functions of these additional modifications of CREB and CBP are only now becoming appreciated, and may not be universal.

to phosphorylated CREB, may require additional modification to be maximally activated, and this lends credence to the possibility that CBP, like CREB, is inducibly modified by stimulation.

The nature of such hypothetical modifications and the extent to which they are necessary for CREB-dependent gene expression are now beginning to be addressed (Figure 6). CBP is phosphorylated in response to stimuli that trigger CREB-dependent gene expression (Hu et al., 1999b; Liu et al., 1999; Zanger et al., 2001), and recently Goodman and colleagues have identified CBP Ser-301 as a CaMKIV substrate that is phosphorylated in cultured hippocampal neurons in response to NMDA receptor activation (Impey et al., 2002). Mutation of this residue to Ala reduces the Ca^{2+}-induced increase in GAL4-CBP-driven reporter gene expression. It is unclear how this phosphorylation event contributes to an increase in CREB-dependent transcription, but one potential mechanism might involve changes in CBP affinity for CREB, or for components of the basal transcriptional machinery. Nonetheless, this observation underscores the possibility that regulatory mechanisms central to stimulus-specific CREB-dependent gene expression may reside, at least in part, at the level of inducible modifications of CBP. Interestingly, phosphorylation appears not to be the only modification of CBP that is relevant to its function as a CREB-transcriptional coactivator. Evans and coworkers have recently demonstrated that CBP is a target of the methylase CARM1, and that CBP modification by arginine methylation within its KIX domain serves to disrupt KID-KIX interactions, and to decrease CREB-dependent transcription, presumably by preventing formation of the CREB-CBP complex (Xu et al., 2001).

Finally, in addition to modification of CBP, the regulation of CREB-dependent gene expression may very well involve modifications of CREB at sites other than Ser-133 (Figure 6). Indeed there are several potential phospho-acceptor sites within CREB (Gonzalez et al., 1989) that have largely remained uninvestigated. While an early study reported that phosphorylation of CREB at Ser-142 can prevent CREB-mediated transcription (Parker et al., 1998), the significance or relevance of this and other CREB phospho-acceptor sites has been revisited only recently. Schütz and colleagues have undertaken a genetic approach to assess the function of Ser-142 in vivo, having observed that it is phosphorylated in the mouse SCN in a manner dependent upon light and circadian time (indeed in a pattern that mirrors that of Ser-133 phosphorylation; Gau et al., 2002). In a knockin mouse harboring a CREB Ser-142Ala mutation, light-induced expression of c-*fos* and *mPer1* in the SCN was attenuated, and light-induced phase shifts of the circadian clock were impaired (Gau et al., 2002).

The mechanisms by which Ser-142 is believed to function in stimulus-dependent gene expression are not speculated in this study, but they have begun to be addressed by Greenberg and colleagues, who have shown that CREB Ser-142 and, in addition, Ser-143 are phosphorylated in cultured cortical neurons in a Ca^{2+}-specific manner (Kornhauser et al., 2002). They demonstrate that phosphorylation of all three Serine residues, 133, 142, and 143, is needed for maximal Ca^{2+}-induced CREB-dependent gene transcription, and, in general agreement with earlier findings (Parker et al., 1998), they show that phosphorylation at these sites prevents CREB-CBP interactions. The observation that Ser-142/143 phosphorylation may disrupt CREB-CBP binding (Kornhauser et al., 2002), yet is required for CREB-dependent gene expression in response to certain stimuli (Gau et al., 2002; Kornhauser et al., 2002) implies the very provocative possibility that some CREB-dependent genes may be expressed if not entirely independent of CBP, at least independent of a direct CREB-CBP interaction.

Perspectives

At the time of the discovery of CREB as a mediator of cAMP-inducible transcription, perhaps no one could

have predicted that in a little over a decade this factor would emerge as a key component of biological processes so numerous and so diverse. CREB is now well established as a critical regulator of responses to many neuronal stimuli, but how, exactly, does it function? The extent to which the outputs of these stimuli may vary is consistent only with a conclusion that CREB and its close relatives function as general mediators of cellular change. The nature of the change must be guided in some way by context-specific instructions imparted onto CREB because many CREB target genes are expressed with at least some degree of context specificity. Indeed, few if any CREB target genes are expressed in patterns that precisely mirror CREB activation. These "instructions" that specify CREB function are only now beginning to be understood. Since it has become clear that CREB phosphorylation on Ser-133 must not and cannot alone activate target gene expression, these instructions must engender multiple levels of regulation. With the recent discoveries of stimulus-induced modification of CREB at multiple sites, and modification of CREB cofactors such as CBP, it is tempting to speculate that the context-specific regulation of CREB-dependent transcription resides in the ability of specific contexts to differentially control these and other regulatory parameters. Further, cell type-specific transcription factors have recently been discovered that support CREB-dependent gene expression, perhaps through facilitating CREB-mediated recruitment of coactivators and components of the basal transcription machinery to specific promoters. One such transcription factor is ACT, which upon association with CREM supports CREM-mediated transcription in the testis (Fimia et al., 1999). Thus, a complex combination of context-specific modifications and cell-type specific transcriptional activators and coactivators could enable CREB to coordinate the many neurobiological processes described here, and undoubtedly others which remain to be identified. Indeed, it seems that in an investigation of any given process, simple or complex, physiological or pathological, one is probably justified in assuming that CREB-dependent gene expression is more likely to be involved than not. Exciting reports over the past several years have identified many such processes that depend upon CREB family members, and ongoing studies promise to unravel the mysterious mechanisms by which these factors embody such splendidly diverse capabilities.

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