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More Than Just an OFF-Switch: The Essential Role of Protein Dephosphorylation in the Modulation of BDNF Signaling Events

Katrin Deinhardt and Freddy Jeanneteau

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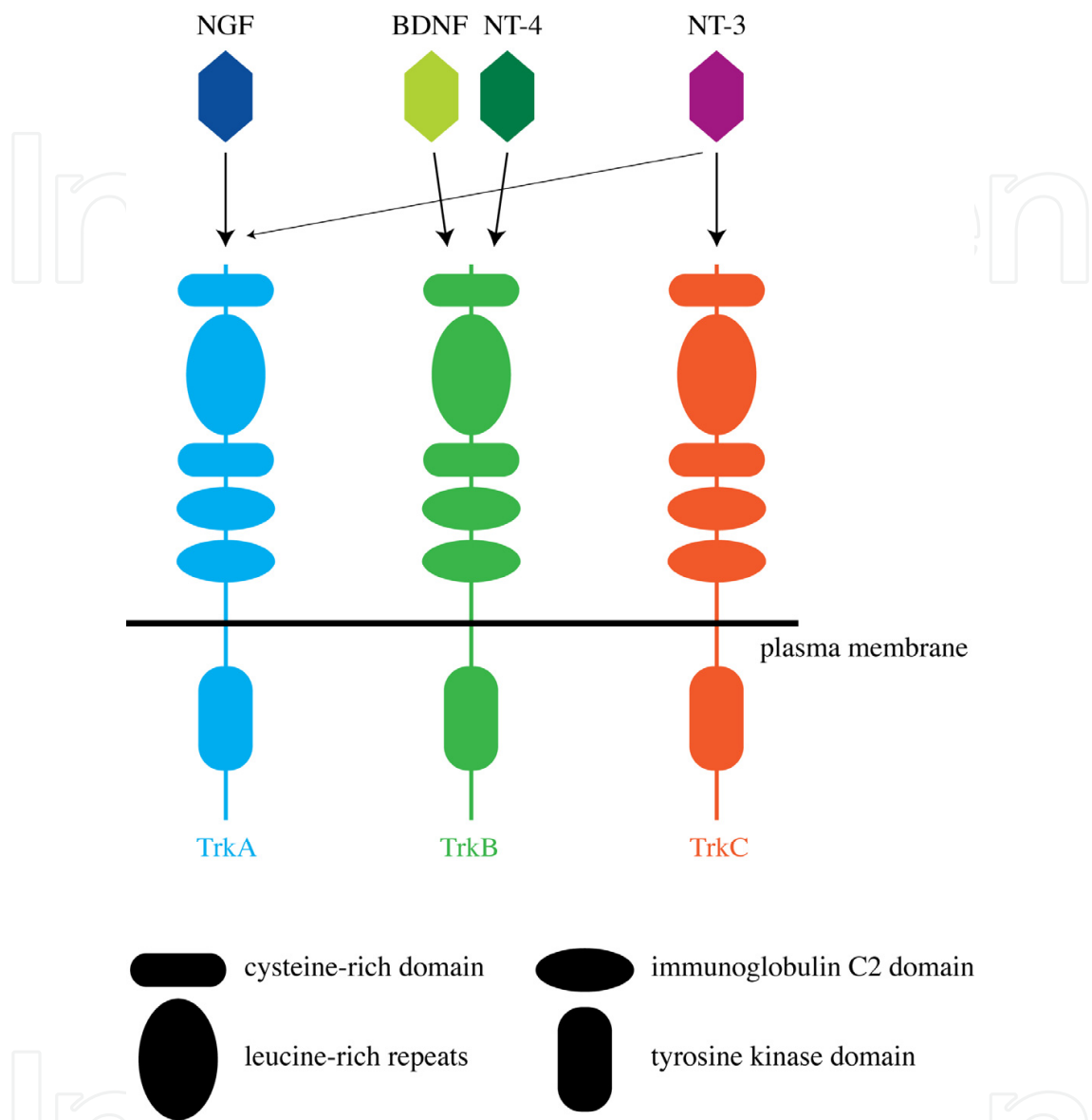
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1. Introduction

Upon binding to their receptors, growth factors trigger intracellular signaling cascades. These cascades are propagated and modulated by many subsequent phosphorylation/dephosphorylation events, which ultimately influence the cellular response. BDNF, a major growth factor of the central nervous system, is a member of the family of neurotrophins, which also comprises nerve growth factor (NGF) and neurotrophins (NT) 3 and 4. Neurotrophins bind to their cognate Trk receptor tyrosine kinases TrkA, B or C, to initiate downstream signaling events (Figure 1) (1, 2). During development, neurotrophins act as target-derived growth factors, which are essential for the survival of selective populations of neurons, especially within the peripheral nervous system (3). However, neurotrophin signaling extends well beyond effects on neuronal survival during development, and also plays important roles in higher order function in the adult, such as behavior, learning and memory. This is best characterized for BDNF- TrkB signaling. Mature BDNF facilitates long-term potentiation and dendritic spine formation in the hippocampus, a process that has been implicated in learning and memory (4). Animals with lower levels of BDNF or its receptor TrkB, as well as mice harboring a common polymorphism in the *bdnf* gene leading to decreased BDNF secretion, give rise to eating disorders and an increase in anxiety-related behaviour (5). Moreover, decreases in BDNF have been correlated with depression while increases in BDNF seem to have an antidepressant effect, and conversely, commonly prescribed anti-depressants raise BDNF levels (5, 6).

In this chapter, we will describe in detail examples of feedback and feed-forward loops downstream of BDNF-TrkB signaling, which transmit and adjust the signal, and therefore determine the ultimate physiological outcome. These cascades comprise multiple protein phosphorylation and dephosphorylation events to modulate both the duration and localization of the signal, as well as the downstream targets affected. These processes help

shed light on how a single, well conserved ligand-receptor pair can have such diverse effects on cellular physiology as described above for BDNF-TrkB.



The neurotrophin family consists of four closely related proteins encoded by four different genes that include NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophin-3) and NT-4 (neurotrophin-4). Neurotrophins act as homodimeric ligands for the Trk (tropomyosin-related kinase) receptors. Three Trk receptors encoded by independent genes afford selectivity among neurotrophins with NGF and NT-3 binding to TrkA, BDNF and NT-4 binding to TrkB and NT-3 binding to TrkC. All Trk receptors are composed of extracellular leucine-rich repeats, cysteine-rich domains and a single immunoglobulin C2 domain that ensure proper conformation of the ligand-binding pocket. Neurotrophin binding promotes homodimerization of Trk receptors, which in turn initiates intracellular phosphorylation cascades. The intracellular tyrosine kinase domain common to all Trk receptors is necessary for neurotrophin signaling as it allows transphosphorylation of several key tyrosine residues of the Trk receptor dimer at the origin of pleiotropic neurotrophic function. Several splice isoforms of the Trk receptors encode for a truncated protein that lack large portions of the intracellular domain. The TrkB.T1 and TrkB.T2 isoforms lack the prototypical tyrosine kinase domain. These isoforms are abundant in the adult brain and notably in glial cells. Nevertheless, glial cells respond to neurotrophin by eliciting distinct intracellular events. For instance, BDNF signaling mediated by the glial truncated TrkB.T1 isoform elevate intracellular calcium and associated phosphorylation waves mediated by calcium-sensing proteins kinases like CaMK2.

Figure 1. Neurotrophins and Trk neurotrophin receptor family of proteins.

2. Immediate changes in protein phosphorylation downstream of BDNF/ TrkB

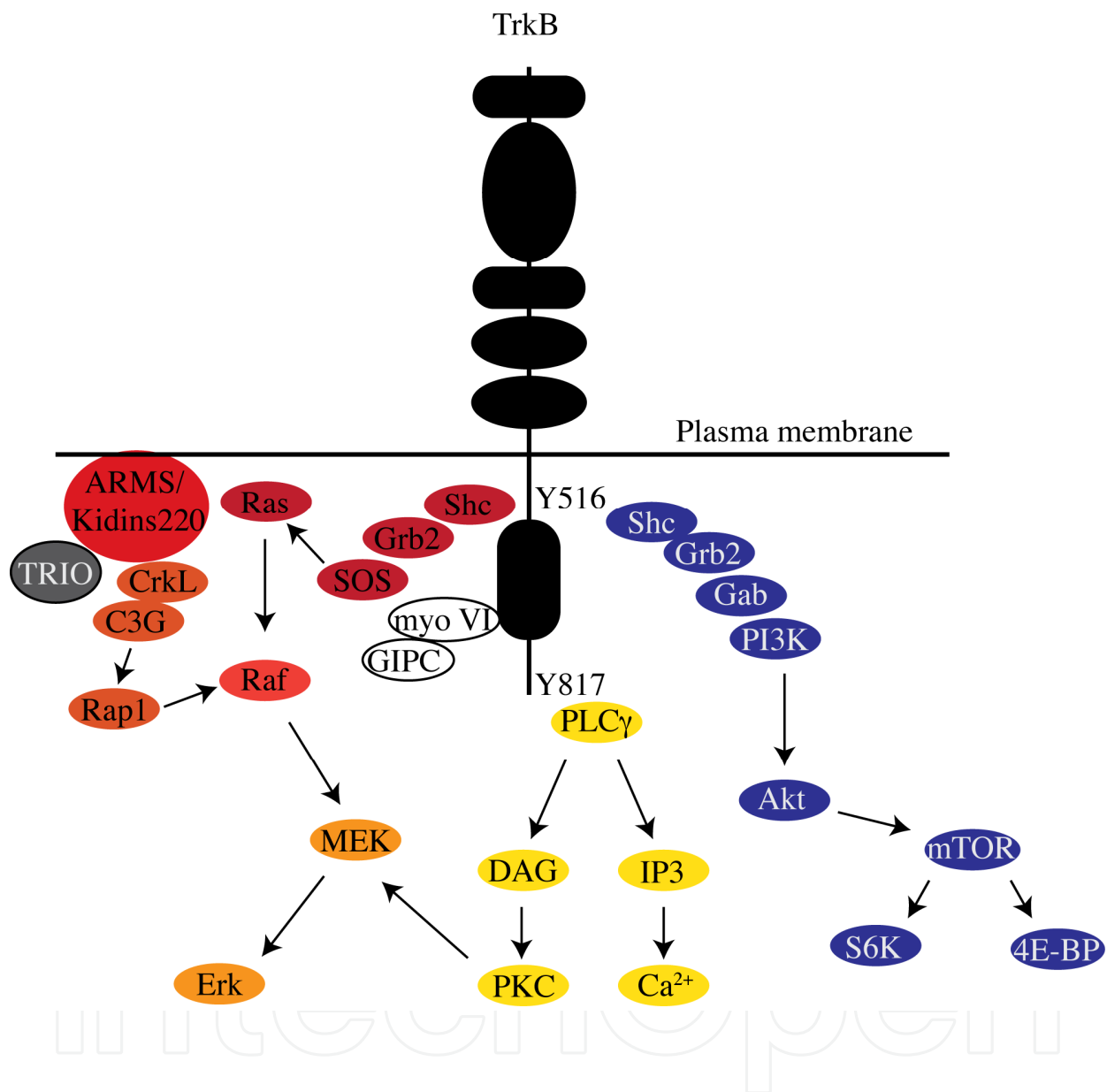
Activation of TrkB upon BDNF binding leads to receptor dimerization and phosphorylation, thereby creating docking sites for effector proteins that initiate the activation of intracellular signaling pathways (7). The phosphorylated tyrosine residues Y516 and Y817 in human TrkB receptor serve as the main docking sites to initiate downstream signaling pathways, such as Src homology 2 containing protein (Shc), Akt, mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (Erk) 1/2 and phospholipase C (PLC) γ (Figure 2) (1, 8). This in turn leads to the activation of various pathways involved in cellular functions that range from initiation of gene transcription and protein synthesis to decisions involved in cell growth and survival. The residues Y702, Y706 and Y707, located within the tyrosine kinase domain, can also recruit adaptor proteins when phosphorylated, including Grb2 and SH2B. At the same time as inducing multiple protein tyrosine, serine and threonine phosphorylation events in diverse proteins, BDNF stimulation may cause a reduction in the phosphorylation of other proteins, such as focal adhesion kinase (9), thereby reducing their activity. In the following paragraph, we will discuss in more detail multiple ways of neurotrophin-dependent activation of Erk1/2 signaling pathways.

3. Multiple cascades leading to BDNF-dependent Erk1/2 activation

Three major pathways mediate activation of Erk1/2 downstream of BDNF: PLC γ / PKC signaling following phosphorylation of Y817 site, or Shc-Grb2 signaling through Ras or Rap1 (Figure 3) (1).

Phosphorylation of hTrkB (human TrkB) at the most C-terminal tyrosine, Y817, leads to the recruitment and activation of PLC γ , which hydrolyses phosphatidylinositol(4, 5)bisphosphate (PI(4,5)P₂) into diacylglycerol (DAG) and inositol tris-phosphate (IP₃). IP₃ subsequently leads to release of intracellular Ca²⁺, which in turn activates Ca²⁺-dependent enzymes such as Ca²⁺-calmodulin-regulated protein kinases (CaM kinases), as well as the phosphatase calcineurin. Additionally, the release of Ca²⁺ and the production of DAG activate protein kinase C (PKC), which stimulates Erk1/2 signaling via activation of Raf and the mitogen activated protein kinase kinase MEK.

Along another pathway leading to active Erk, phosphorylation of hTrkB at the tyrosine residue closest to the plasma membrane, Y516, creates an Shc binding site to initiate downstream transient or prolonged Erk1/2 signaling. Transient activation follows the recruitment of a complex of growth factor receptor bound protein 2 (Grb2) and the Ras activator son of sevenless (SOS), which in turn stimulates activation of Ras and downstream, the activation of the c-Raf/ MEK/ Erk1/2 cascade. Erk in turn phosphorylates SOS, leading to the dissociation of the Grb2/ SOS complex, which then no longer activates the Ras/ c-Raf/ MEK cascade, thus only leading to transient activation of Erk.



Upon binding to BDNF, a series of tyrosine phosphorylation events occur within TrkB cytoplasmic domain. These phospho-tyrosine residues form unique binding sites for intracellular adaptor proteins, Y516 and Y817 recruiting specifically Shc and PLC γ , respectively. The typical wave of second messengers involves, PLC γ , which raises intracellular calcium, the Ras-Erk and PI3-Kinase/Akt pathways. The elevation of intracellular calcium allows the activation of CAMK (calmodulin kinases), which converges with the Erk pathway to activate transcription factors like CREB within the nucleus by phosphorylation on S133. Transcription factors like CREB transform transient BDNF signaling into durable signaling by changing the expression of many selective target genes.

Figure 2. Proximal intracellular signaling of the TrkB receptor.

Prolonged Erk activation is also initiated at the Y516 site, but requires the adaptor protein ankyrin-rich membrane spanning protein (ARMS, also known as Kidins220), which recruits Grb2 and CrkL. This complex formation depends on tyrosine phosphorylation of ARMS at the residue Y1096. Binding to CrkL then activates the Rap exchange factor C3G and thus initiates prolonged Rap1/ Raf-dependent MEK/ Erk1/2 signaling. Accordingly, loss of ARMS impairs Rap1 activation and prolonged activation of Erk1/2, while early Erk1/2 activity is not affected (10).

Ultimately, Erk1/2 signaling not only promotes local axonal growth via cytosolic signaling events, but activated Erk1/2 can also translocate to the nucleus to phosphorylate and activate transcription factors, such as CREB (cAMP response element-binding), leading to the initiation of transcriptional events and the expression of immediate early genes (IEGs), which can further modulate the response (11).

4. The importance of signal duration

In the mid 1990s, it became widely recognized that the actual signal duration will critically influence the ultimate cellular outcome (12). Because the MAPK pathway (e.g. Erk1/2, JNK, P38) is central for the activation of transcription factors, the expression of a large array of genes is sensitive to extracellular trophic factors, such as BDNF (13). One classical example that highlights the importance of the temporal control of signaling is the activation of Erk1/2 downstream of NGF versus EGF in pheochromocytoma (PC12) cells (14). NGF leads to sustained activation of Erk1/2 by inducing a positive feedback loop on to the upstream activator Raf, whereas EGF activates a negative feedback loop to Raf and thus leads to transient activation of Erk1/2. As a consequence, EGF promotes cell division, while NGF initiates differentiation. This is not specific to PC12 cells, as sustained Erk1/2 activity also transforms fibroblasts and macrophages (15, 16). Indeed, under pathological conditions unrestricted activation of the Ras-Erk1/2 pathway through point mutations is one of the most frequently identified cellular defects leading to tumour formation and cancer (17).

One explanation for a differential duration of Erk1/2 activation downstream of different growth factors may be the expression levels of their cognate receptors. Indeed, PC12 clones with low amounts of the NGF receptor TrkA fail to differentiate in response to NGF, while PC12 cells overexpressing EGF receptor can undergo differentiation in response to EGF (18). In line with this, tumour cells frequently upregulate growth factor receptors, leading to aberrant activation and transformation (19).

In addition to activation through distinct cascades, both positive and negative feedback loops further influence the amplitude and duration of MAPK signals. For example, BDNF induces its own release, thus initiating further rounds of receptor-mediated signal activation (20). On the other hand, Erk1/2 phosphorylates SOS, which impairs SOS-Grb2 complex formation and thus decreases further activation of Erk1/2 (21). Moreover, multiple phosphatases (PP1, PP2a and MKPs) inactivate ERKs, implying that the duration

and extent of ERK activation is controlled by the balanced activities of the upstream ERK kinases, MEKs, as well as MEK and ERK phosphatases (22-24). Modeling approaches showed that these multiple layers of feedback along the MAPK cascade can not only determine the strength and timing of the signal, but also induce robust and sustained signal oscillations, thereby ultimately allowing for a multitude of biological outcomes (25).

5. Localizing the signal: Signal transduction cascades versus trafficking and intracellular localization of the ligand-receptor complex

Receptor tyrosine kinases are activated by ligand binding at the cell surface. They are subsequently internalized into the endosomal pathway and either recycled back to the plasma membrane for further rounds of activation, or sorted to the lysosome for degradation. For a long time, the classical belief was that signal transduction terminates with receptor internalization. However, receptors are internalized very rapidly upon activation, thus leaving only a short time window to induce signal transduction. In the mid 1990s the view changed and it became accepted that receptors continue to signal following internalization, from so-called signaling endosomes (26-28). Much work has since focused on endosomes as signaling platforms, and it is now well established that cascades such as the Erk1/2 or Akt pathways can be initiated from endosomal membranes. For example, the MEK1 scaffold, MEK partner 1 (MP1), recruits MEK1 and Erk1/2 to endosomal membranes, and knockdown of MP1 leads to lack of recruitment and a concomitant reduction in Erk1/2 signaling (29).

The essential role of signaling from endosomes in physiological processes such as polarization and migration is widely accepted. In addition, a large body of work has highlighted the importance of signaling endosomes in neurons, where signals at times have to traverse large distances. For example, intracellular trafficking of the signal-receptor complex from the synapse along the axon and to the cell body is essential for health and survival of neurons of the peripheral nervous system, which depend on target-derived growth factors (30, 31). Consequently, mutations in genes involved in this retrograde axonal transport process are frequently associated with neuropathies (32).

Interestingly, two recent studies in cell lines questioned the view that intracellular trafficking of the activated ligand-receptor complex is critical for downstream cellular responses (33, 34). Following EGF stimulation, they demonstrated that the cascades eventually influencing transcriptional changes are already initiated at the plasma membrane, and within a short time after ligand binding. Consequently, interfering with subsequent ligand-receptor sorting had only minor effects on the overall transcriptional response. How these findings apply to different cell systems, for example in highly polarized cells such as neurons, remains to be seen. At least for peripheral neurons, it has been demonstrated that internalization of the NGF-TrkA complex at the distal axon is strictly required for activation of CREB, initiation of transcription, and survival (28).

6. Activation of an essential CREB co-activator through dephosphorylation

Phosphorylation of CREB is an activation mark that is required but not sufficient to induce the expression of responsive-genes (35). The CREB co-activator, transducer of regulated CREB (TORC or CRTC) must be actively transported to the nucleus to allow CREB-dependent transcription of target genes (35). There are three members of the CRTC family (CRTC1/2/3) that are encoded by independent genes. Each isoform presents specific expression profile and binding to CREB-occupied genes, but redundancy was highlighted by loss-of-function experiments (36). For instance, CRTC1 null mice decrease BDNF expression in the prefrontal cortex and hippocampus down to 40 % of wildtype levels, suggesting additional mechanisms of regulation and/ or compensatory mechanisms (37). Knockdown of CRTC1, the major isoform in the limbic brain is sufficient to prevent BDNF-induced structural plasticity such as dendritic and axonal arborization, as well as physiological features like long-term potentiation that also depends on BDNF/TrkB signaling (38, 39). Because the transcriptional activity of CREB downstream of BDNF depends on CRTC1 and perhaps other CRTC isoforms, it is now interesting to describe how CRTC1 function is regulated.

When phosphorylated, CRTC proteins reside in the cytoplasm of resting neurons via high affinity interactions with 14-3-3, a family of cytoplasmic scaffold proteins that help organize and sequester phosphorylated proteins in the cytoplasm. Upon neuronal activation, CRTC proteins become dephosphorylated notably at residues S171, S275 and S307, which unmask a nuclear localization sequence and permit the translocation of CRTC proteins into the nucleus (40). If at the same time CREB is phosphorylated, the formation a CREB-CRTC1-CBP protein complex can drive transcription at target genes (Figure 3). Such a mode of action raises questions about the coincidence detection mechanisms that allow the convergence of CREB phosphorylation and CRTC dephosphorylation. Calcineurin, a calcium sensing phosphatase, directly dephosphorylates CRTC proteins. BDNF signaling induces robust and sustained phosphorylation of CREB, but cannot elicit nuclear translocation of CRTC1 (38). In similar experiments conducted in hypothalamic neurons, where CRTC2 is the major isoform expressed, BDNF signaling did not elicit CRTC2 translocation to the nucleus, while cAMP-elevating drugs or drugs that elevate intracellular calcium did (41). Although BDNF/TrkB signaling has been previously shown to elevate cAMP and calcium levels in neurons (1, 42), it is believed to occur and remain local, such as neurite terminals (20). However, it is clear that elevation of intracellular cAMP or calcium facilitate the functional and morphological synaptic responses to BDNF signaling (43, 44). Elevation of intracellular calcium activates calcineurin whereas cAMP activates PKA, which in turn phosphorylates and deactivates the upstream CRTC kinase, salt-inducible kinase (SIK). Only such coordinated signaling guarantees efficient translocation of CRTC proteins to the nucleus (Figure 3). So, what neuronal mechanisms mediate calcineurin-dependent dephosphorylation of CRTC protein? Neuronal activity via calcium-permeable glutamate ion channels not only phosphorylates CREB, but also triggers the calcineurin-dependent dephosphorylation of CRTC1 in cortical neurons and of CRTC2 in hypothalamic neurons that is necessary to activate CREB-mediated transcription (38, 41).

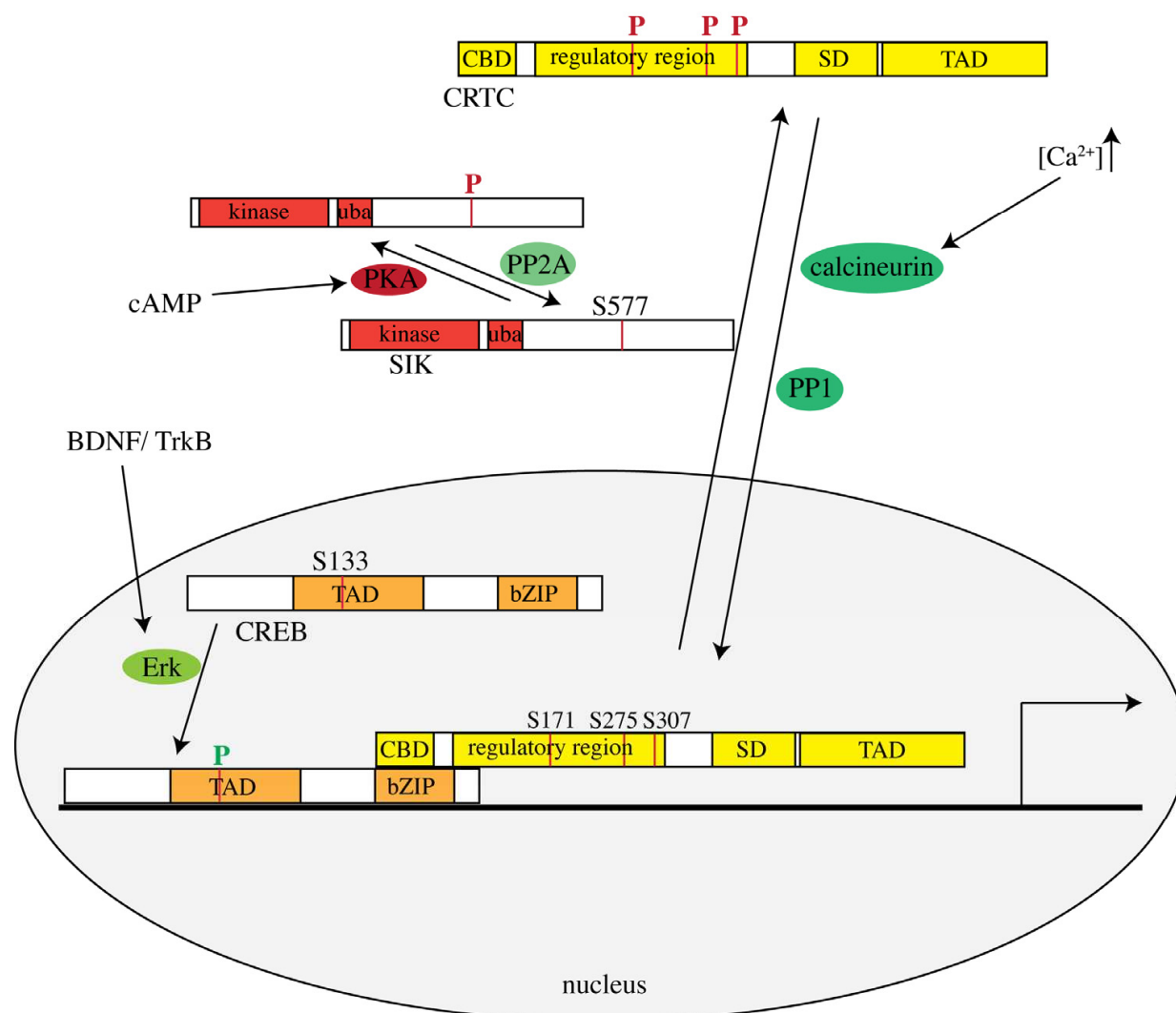


Figure 3. Subcellular compartmentalization of CRTC proteins regulates their function as necessary CREB-cofactors. CRTC proteins are constitutively phosphorylated in many cell types including neurons. Consequently, they reside in the cytoplasm via sequestration by the 14-3-3 family of proteins. When actively dephosphorylated by the calcium-sensing phosphatase calcineurin, a nuclear localization sequence is unmasked and CRTC translocates to the nucleus. Efficient translocation of CRTC protein to the nucleus is also governed by concomitant inactivation of the CRTC upstream kinases like SIK (Salt-inducible kinase) via its phosphorylation by the protein kinase A. Once in the nucleus, CRTC proteins form a multi-complex with the phosphorylated form of CREB, thus, acting as a permissive signal for robust and specific genomic signaling response.

7. Wait a minute: Immediate-early gene expression following BDNF stimulation

Following the immediate phosphorylation and dephosphorylation events, BDNF signaling eventually results in a nuclear response and immediate-early gene (IEG) expression. This process starts within minutes, and the first translated proteins are detectable within 30 min following the initial stimulation. Many IEGs are themselves transcription factors, such as fos, which will set off a series of transcription/ translation waves to adjust the cellular

transcriptome and proteome. Other IEGs directly modulate cellular shape and responsiveness. The probably best-characterized BDNF-sensitive IEG is Arc (Arg3.1), a modulator of actin depolymerizing factor (ADF)/ cofilin activity and regulator of AMPA receptor trafficking (45). Local translation of Arc plays an essential role in the consolidation of long-term potentiation, which equates to an increase in synaptic transmission in response to neuronal activity. In addition, BDNF initiates its own synthesis and release, thereby strengthening and prolonging its own signal and starting a positive feedback loop. Other IEGs induced by Erk1/2 signaling are phosphatases, which provide a negative feedback loop. For example, we recently reported that BDNF-TrkB signaling induces the expression of the dual-specificity phosphatase MKP-1 (MAP kinase phosphatase 1, also known as DUSP1) (46). MKPs comprise a family of phosphatases that recognize and dephosphorylate both the threonine (T) and tyrosine (Y) within a TxY motif in the catalytic core that is conserved among the family of MAPKs (24). Below, the role of MKP-1 expression in modulating MAPK signaling, and its ultimate cellular consequences, will be discussed in greater detail.

8. MKP-1 molds the MAPK signal and neuronal morphology

Basal MKP-1 protein levels within the cell are very low, but its expression is induced following Erk1/2 activation by growth factors in a variety of cell types (47). Generally, MKPs have a preference for specific members of the MAPK family, and the favored substrates for MKP-1 are JNK, p38 and Erk1/2. Therefore, MKP-1 can terminate the signal that initially induced its expression (24). This form of feedback inhibition is a common theme in many signal transduction events, such as discussed above for Erk1/2 signaling. After induction, MKP-1 is rapidly degraded by the proteasome within one hour post stimulation. But BDNF signaling prolongs MKP-1 protein stability by several hours (46). Erk1/2-dependent phosphorylation of carboxy-terminal serine residues can modulate the half-life of MKP-1 protein. One study proposed that phosphorylation of serines 296 and 323 is required for recognition of MKP-1 by the ubiquitin ligase, thereby promoting degradation (48), while another study suggested that phosphorylation at serines 359 and 364 interferes with ubiquitination and thus prevents degradation (49). Serial mutagenesis of such signature motifs helped crack MKP-1 phosphorylation code (Figure 4). Only the mutations of all four phospho-sites alter significantly MKP-1 protein half-life whereas mutations of individual phospho-sites or motifs (S296/S323 and S359/S364) are mainly ineffective. Notably, alanine mutations in place of serines 359 and 364 as well as phospho-mimicking glutamate mutations in place of serines 296 and 323 in a single quadruple mutant diminish MKP-1 protein stability despite BDNF signaling. Therefore, BDNF signaling not only induces *mkp-1* expression but also prolongs MKP-1 protein half-life as a result of phosphorylation/dephosphorylation of MKP-1 C-terminal signature motifs (Figure 4) (46). If the kinase involved in MKP-1 phosphorylation at serine 359 in neurons is Erk1/2, the identity of the phosphatases for serines 296 and 323 are presently unknown.

Within the immune system, MKP-1 is largely nuclear. One of its best-described functions is terminating cytokine production and therefore the immune response by inactivating p38 and p38-mediated transcription following an immune challenge. Indeed, mice lacking

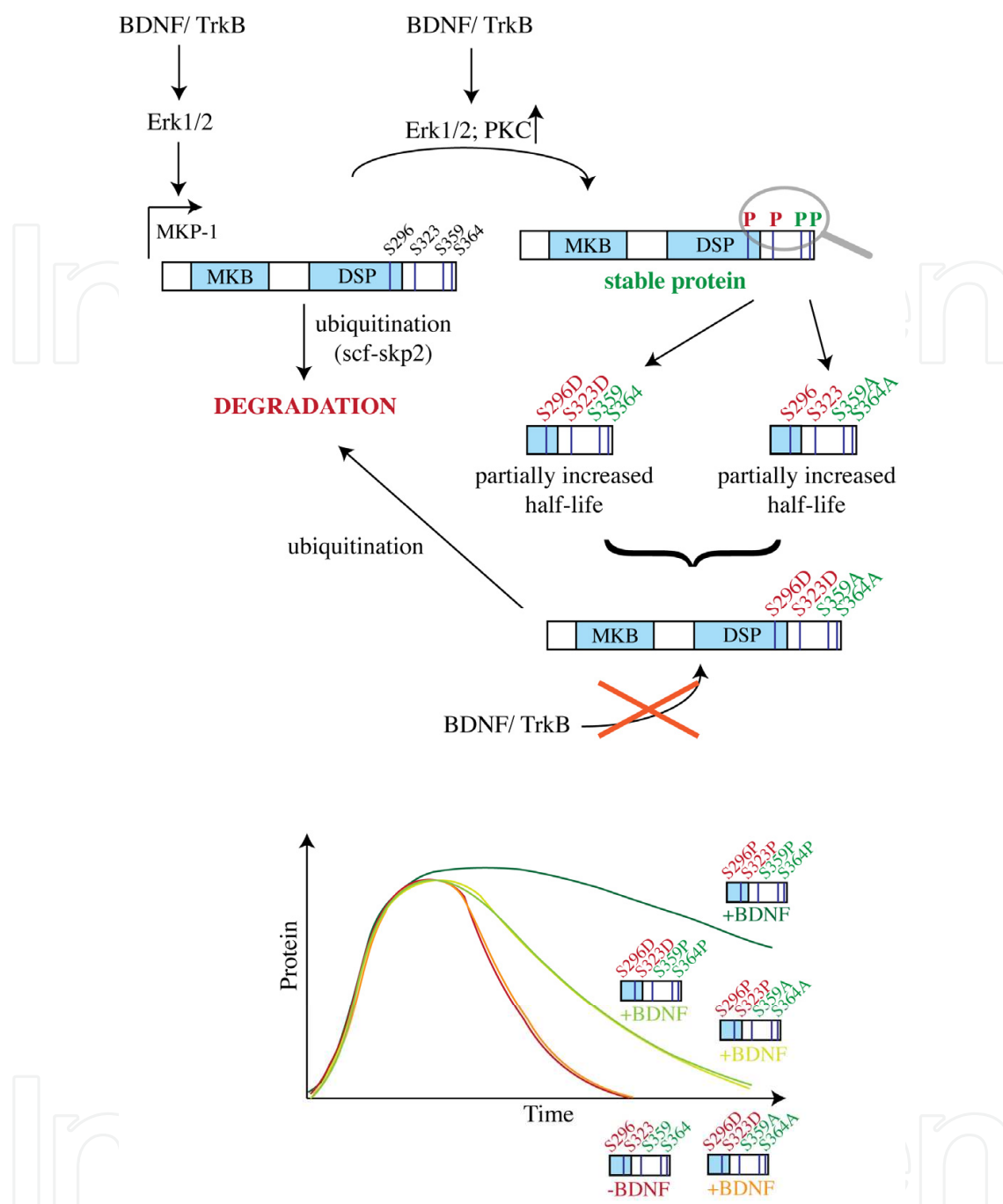


Figure 4. *Mkp-1* is a BDNF and CREB sensitive gene. Among the many genes regulated by CREB, *mkp-1* is an immediate early gene that can be expressed within minutes of BDNF stimulation. With low basal expression in resting neurons, the induction of the *mkp-1* gene product represents a mechanism of activation necessarily delayed in time from the initial BDNF signaling cascade. It is thought that MKP-1 protein serves to terminate the MAPK signaling via a negative feedback loop mechanism, since MKP-1 is a deactivating MAPK phosphatase. To support this view, the protein turnover of MKP-1 is extremely rapid (< 1 hour) allowing the rapid reinstatement of basal MAPK signaling homeostasis. Of note, phosphorylation of MKP-1 by Erk is sufficient to stabilize the protein. This serves to maintain MKP-1 function where needed in the cell whereas other signaling compartments lacking Erk signaling are preserved. So, MKP-1 induction and stabilization offers a sophisticated mode of regulation for MAPK signaling in time and space.

MKP-1 die due to cytokine overproduction after activation of the immune system (50). In neurons, MKP-1 localises to the cytosol. Here, it inactivates JNK, which is constitutively active within axons of neurons of the central nervous system (51). Prolonged MKP-1 expression thus leads to decreased phosphorylation of JNK substrates. Among the neuronal JNK substrates are many cytoskeleton-associated proteins, such as neurofilaments, tau and stathmins (46). The role of neurofilament phosphorylation is still debated, but has been suggested to influence its axonal transport rate and therefore subcellular localization. Tau has an exceptionally complex phosphorylation signature, and its over 30 phosphorylation sites are affected by many different kinases and phosphatases (52). Under physiological conditions, tau binds to and stabilizes existing microtubules and promotes microtubule growth, but pathological hyperphosphorylation leads to self-assembly of tau, which results both in tau tangles and also in microtubule detabilization. In contrast, the role of phosphorylation on stathmin activity is much better elucidated. Stathmins have four phosphorylation sites, all of them serine residues (53). When phosphorylated at these residues, stathmins are inactive, and dephosphorylation therefore equates to activation. Active stathmins sequester free tubulin dimers, the building blocks for microtubules, and therefore passively decrease microtubule growth (53). This results in an increase in microtubule dynamics or a decrease in microtubule stability.

Spatio-temporal control of MKP-1 expression is critical to increase dynamic microtubules, a prerequisite for the remodeling of the cytoskeleton that manifests in the forms of branching or pruning of axonal collateral arbors. For instance, transient expression of MKP-1 permits branching of axons (46). On the other hand, disruption of such spatio-temporal expression of MKP-1, in the forms of ectopic expression or sustained protein half-life over prolonged periods of time impedes axonal growth or prunes axons, depending on the developmental window.

9. From behavior to MKP-1 and back

Arborization of axons is critical to adjust neural network wiring to environmental needs. Behavioral experience evokes multiple signaling pathways that converge to cytoskeletal remodeling via both slow genomic and rapid non-genomic mechanisms. Protein phosphorylation is instrumental in the signaling toolbox that controls both of these programs. The balance of phosphorylation and dephosphorylation of the molecular components of the cytoskeletal machine dictates, in part, its continuous plasticity (54). By challenging this equilibrium at defined subcellular locations, timely, BDNF signaling instructs the position of branching points that mold the topographic maps of innervation. The manifold aspects of phosphorylation on BDNF signaling in this process can be illustrated by the molecular mechanisms that regulate the expression, localization and degradation of MKP-1, the dual specificity MAPK phosphatase. Induction of MKP-1 requires both a linear phosphorylation cascade through the MAPK-ERK1/2-CREB pathway and a feed-forward dephosphorylation switch via the calcineurin-CRTC1/2 pathway (Figure 5). Failure of either signaling branches prevents *mkp-1* expression, which impinge on activity-dependent remodeling of the cytoskeleton. Baseline *mkp-1* mRNA expression is limited in the brain of naturally behaving songbirds and rodents (55, 56). But hearing song, seeing visual stimuli, or performing motor behavior robustly increases *mkp-1* transcripts respectively, in auditory, visual, and

somatosensory neurons (56-58). Therefore, expression of *mkp-1* is driven by neural activity in the primary sensory areas of the brain. What may result from the deregulation of MKP-1 expression during higher order behaviors? When ectopically expressed in humans and animal models, MKP-1 triggers depressive behavior (55). In contrast, genetic disruption of *mkp-1* protects from stress in an animal model (55). Therefore, too little MKP-1 may prevent activity-dependent remodeling of axons and dendrites that is necessary for behavioral adaptation, learning and memory. In contrast, too much MKP-1, as observed in depression and stress, may destabilize cytoskeletal architecture that impinges on the maintenance of functional neural networks. How titration of MKP-1 levels affects structural characteristics such as dendritic spines, physiological features like long-term potentiation and cognitive performance like learning and memory is unknown. Future investigations will shed light into these possibilities.

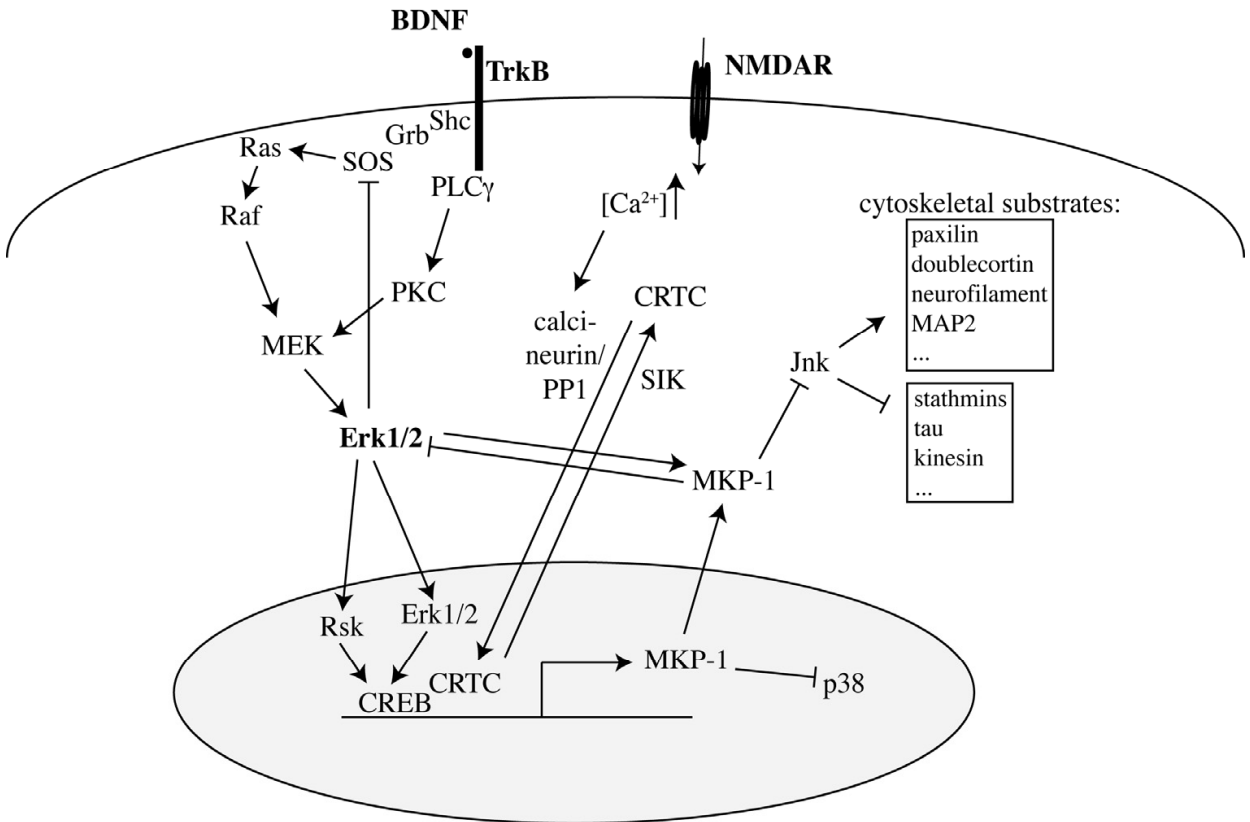


Figure 5. BDNF signaling from the plasma membrane to the nucleus. Activation of TrkB by BDNF rapidly elicits numerous waves of protein phosphorylation that converge to the nucleus to trigger a genomic response. Coincidence detectors like CRTC proteins may determine the nature of the genomic response based on the cellular context. In effect, phosphorylation of CREB by BDNF signaling is required but not sufficient to elicit a transcriptional response. Neuronal activity and excitatory neurotransmitter, glutamate signaling converge to CREB activation via the dephosphorylation and nuclear translocation of the CREB-coactivators, CRTC. Among the regulated genes, MKP-1 is a MAPK phosphatase that terminates upstream BDNF-induced signaling in a delayed fashion. But MKP-1 serves also to deactivate JNK, which is constitutively activated in the axon. In consequence, many of the JNK substrates that serve cytoskeletal attributes are dephosphorylated. Numerous cytoskeletal-binding proteins that are substrates of JNK are either activated or deactivated upon phosphorylation. Spatio-temporal phosphorylation and dephosphorylation of these proteins helps maintain the integrity of the cytoskeletal architecture of the axon, as much as its remodeling capacity.

10. Conclusion

We have described how multiple steps of phosphorylation and dephosphorylation along the signaling pathways initiated by the neurotrophin BDNF determine its function via its receptor TrkB. These phosphorylation and dephosphorylation loops serve to efficiently activate in time and space the downstream components of BDNF signaling either proximal to the plasma membrane or distal in the nucleus. Concurrent activation of a kinase and deactivation of the substrate's phosphatase is a common, but not exclusive mechanism to ensure both the robustness and fidelity of the output signal. Indeed, phosphorylated or dephosphorylated residues may serve to accelerate or delay the target protein's turnover as exemplified by MKP-1. Where BDNF signaling is not capable of initiating its entire signaling machinery such as the dephosphorylation of CRTC proteins, it relies on converging signaling pathways. Coincidence detectors, like CRTC proteins may serve to 'gate' neurotrophin signaling and filter the physiological output consequences as a function of the cellular context.

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