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Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Induced Gene Regulation in Brain

A Molecular Substrate for Learning and Memory?

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Abstract

The mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) pathway is an evolutionarily conserved signaling cascade involved in a plethora of physiological responses, including cell proliferation, survival, differentiation, and, in neuronal cells, synaptic plasticity. Increasing evidence now implicates this pathway in cognitive functions, such as learning and memory formation, and also in behavioral responses to addictive drugs. Although multiple intracellular substrates can be activated by ERKs, nuclear targeting of transcription factors, and thereby control of gene expression, seems to be a major event in ERK-induced neuronal adaptation. By controlling a prime burst of gene expression, ERK signaling could be critically involved in molecular adaptations that are necessary for long-term behavioral changes. Reviewed here are data providing evidence for a role of ERKs in long-term behavioral alterations, and the authors discuss molecular mechanisms that could underlie this role.

Index Entries: MAPK/ERK; long-term memory; MEK inhibitors; synaptic plasticity; gene regulation.

Introduction

Cognitive functions, such as learning and memory formation, but also responses to addictive drugs, require neuronal adaptations in specific brain areas. These adaptations require both strengthening of pre-existing synapses for short-term memory (SM), and growth and maintenance of new synaptic connections for long-term memory (LTM) *(1)*. Although STM depends primarily on the modification, e.g., phosphorylation, of pre-existing cellular proteins, LTM requires alteration in gene expression and synthesis of new proteins

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(2). Complex biochemical processes seem to be involved in activity-dependent synaptic plasticity. Over the past few years, either pharmacological or genetic experiments have demonstrated a role in the process of LTM formation for the Ras-controlled extracellular signal-regulated kinases (ERKs) *(3–6).*

ERKs belong to the family of mitogen-activated protein kinases (MAPKs) that also includes the c-Jun N-terminal kinase (JNK) and p38 members. ERKs are strongly activated by mitogens, and, in the central nervous system (CNS), by neurotrophins and neurotransmitters *(3);* SAPK/JNK and p38 MAPK are induced by various cytokines and stresses, such as osmotic shock, UV irradiation, and excitotoxicity *(7,8).* These Pro-directed Ser/Thr kinases are major effectors of signal transduction from the cell surface to the nucleus. JNK and p38 MAPK mediate the cellular response to stress, and, in many cases, are involved in apoptosis *(9,10),* and ERKs are implicated in cell growth and differentiation, as well as in many functions in differentiated cells.

Studies carried out predominantly in non-neuronal cell lines have characterized what could be considered as the archetypal ERKs cascade *(11,12).* This involves the activation of the Ras family of guanosine triphosphatases (GTPases) via the translocation of guanine exchange factors to the membrane by various adapter proteins that bind specifically to phosphotyrosine residues on the cytoplasmic tails of receptor tyrosine kinases. In neuronal cells, activation of Ras is mediated by a variety of receptor systems, including receptor tyrosine kinase for peptide factors, G-protein-coupled receptors (GPCRs) for neurotransmitters, and calcium (Ca^{2+}) influx through voltage-sensitive Ca^{2+} channels (VSCCs) or N-methyl-D-aspartate receptors (NMDARs) for glutamate *(3).* Ras activates, by an as-yet-unknown mechanism, the MAPK-kinase-kinases (MAPKKKs) of the Raf family. Raf activates the MAPK-kinase (MAPKKs), or MEK1/2, by phosphorylation, which in turn activates ERKs by dual phosphorylation of their TEY motif *(13).*

ERK1 (44 kDa) and ERK2 (42 kDa), which are encoded by different genes, are expressed at high levels in the developing and adult CNS *(14,15).* In neuronal cells, they are present in cytoplasmic compartments, including dendrites, prior to their stimulation *(16).* Once activated, they can phosphorylate a wide range of substrates in the cytoplasm, but also in the nucleus, where they activate transcription factors, and thereby control gene expression, which may be relevant on their role in long-term neuronal adaptations.

By controlling a prime burst of gene expression, ERK signaling could be critically involved in molecular adaptations that are necessary for long-term behavioral changes. The authors review here data providing evidence for a role of ERKs in model systems of long-term behavioral alterations, and discuss molecular mechanisms that could underlie this role.

ERK Signaling and Behavior

A considerable body of literature implicates the Ras/ERK signaling cascade in memory formation. Inhibiting this pathway, using genetic or pharmacological approaches, blocks long-term behavioral changes, such as learning and memory *(6),* but also long-term behavioral changes induced by addictive drugs (*17,18;* Table 1). The first in vivo evidence of ERK implication in mammalian associative learning came from Sweatt's group *(20).* Using a model system of emotional learning (cue and contextual fear conditioning), they showed activation of several kinases, including protein kinase C (PKC), α calcium/calmodulin-dependent kinase II (αCaMKII), and ERK1/2 in the hippocampus, which were dependent on glutamatergic transmission via NMDARs. These learning paradigms were strongly attenuated when SL327, a selective inhibitor of MEK, was injected systemically, prior to, but not after, the conditioning phase, suggesting that ERK1/2 activation was required for the formation of memory, but was nonessential for the ongoing maintenance of memory *(20,21).* More recently, Schafe et al. *(27)*

Table 1

Brain region	MAPK component	Behavioral task	Mode of inhibition	Observed effects	Refs.
Hippocampus	ERK1/2	Morris water maze (rats)	Local injection of PD98059 (BT), without altering acquisition	Blockade of spatial LTM.	*19*
	ERK1/2	Fear conditioning (rats and mice)	Systemic injection of SL327 (BT). cue fear conditioning.	Blockade of contextual and	*20,21*
	ERK1/2	Morris water maze (mice)	Systemic injection of SL327 (BT).	Blockade of spatial learning.	*21*
	ERK1/2	One trial avoidance (rats)	Local injection of PD98059 (AT).	Impairment of STM and LTM.	*22*
	NF1	Morris water maze Fear conditioning	Targeted inactivation of Nf1 gene (heterozygous mice).	Alteration of spatial learning without effect on fear conditioning.	*24*
Insular cortex	ERK1/2 JNK1/2	Conditioned taste aversion (rats)	Local injection of PD98059 (BT).	Attenuation of LTM taste aversion without alteration of STM.	*25*
Entorhinal and parietal cortex	ERK1/2	One trial avoidance (rats)	Local injection of PD98059 (AT).	Impairment of retention.	*23,26*
Amygdala	ERK1/2	One trial avoidance (rats)	Local injection of PD98059 (AT).	Impairment of retention.	*23*
	ERK1/2	Auditory fear conditioning (rats)	Local injection of U0126 (BT).	Impairment of LTM without alteration in STM.	*27*
	Ras-GRF	Fear conditioning Morris water maze	Targeted inactivation of RasGRF gene (homozygous mice).	Impairment of LTM, without alteration in STM and learning. No deficit in spatial learning tasks.	*28*
VTA	ERK1/2	Acute and sensitized locomotor Response to cocaine (rats)	Local injection of PD98059.	Prevents sensitization to cocaine, without altering acute response.	*17*
Dorsal and ventral striatum	ERK1/2	Acute locomotor response and place preference ind uced by cocaine (mice)	Systemic injection of SL327.	Prevents place preference induced by cocaine; poorly affects acute locomotor response.	*18*

AT, after training; BT, before training.

reported a transient activation of ERK in the lateral nucleus of amygdala, a structure classically associated with emotional learning, in an auditory fear conditioning test. Pharmacological blockade of ERK activation, by intra-amygdala infusion of PD98059 or U0126, impaired LTM fear conditioning, without affecting STM *(27,29).* Mice deficient for Ras-GRF, a neuron-specific guanine-exchange factor involved in Ras activation, also exhibit an altered LTM for fear conditioning whereas immediate learning and STM are unchanged *(28).*

Additional evidence comes from Walz et al. *(22,23,26),* who pointed out the complexity of the memory processes, by showing that one-trial, step-down, inhibitory avoidance task (another emotional-learning task) requires a time- and structure-dependent ERK activation. Using local injection of the MEK inhibitor, PD98059, they demonstrated that ERK activation in several brain structures (hippocampus, amygdala, and entorhinal and parietal cortex), was necessary for long-term, but not for short-term, retention of inhibitory avoidance. In this model, NMDARs were required for ERK activation, at least in the hippocampus, since intrahippocampal injection of the NMDAR antagonist, APV, blocked both ERK activation and the inhibitory avoidance responses *(30).*

Spatial learning tasks, which classically involve the hippocampus, are evaluated by the Morris water-maze test. Selcher et al. *(21)* recently reported that blocking ERK1/2 activation, by systemic administration of SL327, impairs this spatial learning performance, without interfering with motivation or motor abilities. Given the potential inhibitory effect of SL327 in the whole brain, a possible involvement of ERK1/2 in extrahippocampal areas, during spatial learning tasks, could not be excluded from these works. The anatomical specificity of ERK1/2 activation, during the Morris water-maze task, was addressed by Blum et al. *(19),* who demonstrated that local infusion of PD98059 in the CA1/CA2 subfield of the dorsal hippocampus, prior to training phase, blocked ERK phosphorylation and attenuated the expression of LTM, without affecting the acquisition phase (STM). Studies using genetically modified mice have also suggested the involvement of this cascade in spatial learning tasks. Neurofibromatosis type 1 (NF1) is a neuronal Ras-GTPase-activating protein, which is thought to negatively control the levels of ERK activation. However, heterozygous mice for NF1 display a spatial impairment in the Morris water maze, but the fear conditioning tasks are unaltered *(24).* In contrast, Ras-GRF mutant mice do not reveal major deficits in the Morris water maze. Thus, interfering with different components of the Ras/ERK cascade differentially impairs spatial and emotional learning *(28).* Finally, Berman et al. *(25)* have reported an activation of ERKs specifically in the insular cortex during conditioned taste-aversion learning. The microinjection of PD98059 in the insular cortex impaired LTM, without affecting STM or sensory, motor, and motivational faculties required for this task.

Although the involvement of ERK signaling in classic learning tasks has been extensively investigated, recent evidence suggests that this pathway can also play a major role in behavioral responses related to addictive processes, such as psychomotor sensitization and conditioned place preference. These two behavioral paradigms, which are highly dependent on context and environmental factors, require associative, as well as non-associative, learning processes. Injection of PD98059 into the ventral tegmental area a dopaminergic (DA) brain structure closely related to drugs of abuse rewarding effects, blocked cocaine-induced behavioral sensitization, without altering acute response to cocaine in rats *(17).* ERK activation, occurring after chronic cocaine administration in the ventral tegmental area was thought to result from stimulation of tyrosine kinase receptors by neurotrophins such as brain-derived neurotrophic factor *(31)* or NT3 *(17).* More recently, results coming from the authors' laboratory have supported the hypothesis that ERKs may be preferentially involved in cocaine-induced, behavioral

rewarding effects, rather than acute effect. Indeed, blockade of ERK activity, by systemic administration of SL327, prevented cocaine-induced conditioned place preference, as well as locomotor sensitization, with only a slight alteration of hyperlocomotion induced by acute cocaine *(18).* In this model, ERK activation occurred in the striatum, a major cerebral target of DA input, and was linked to D1 receptor stimulation, with a partial contribution of NMDARs and D2 receptors. These data are interesting if one considers that most drugs of abuse induce DA release in the striatum *(32,33).* Thus, besides the well-established role of PKA and CRE-binding protein (CREB) in cocaine-rewarding effects *(34,35),* and morphine withdrawal *(36–38),* the authors propose that ERK could also participate to the instatement of long-term neuronal adaptation required for development of addiction.

Taken together, these data strongly support the contention that ERK plays an important role in long-term, rather than short-term, behavioral adaptations.

ERK and Synaptic Plasticity

Long-term potentiation (LTP) is an activity-dependent strengthening of synaptic efficacy, which is generally considered as a cellular model of memory consolidation in mammalian brain. This model has been extensively characterized in the hippocampus, using both in vitro (brain slices) and in vivo approaches. Although multiple kinase cascades, including PKA, PKC, CaMK, have been involved in the mechanisms of LTP induction *(39–42),* several recent studies also implicate ERK1/2 in this form of synaptic strengthening.

Using brain slices, English and Sweatt *(43)* showed that tetanic stimulation (multiple trains of high frequency 100 Hz) of the Schaffer collaterals, which induces NMDARs-dependent LTP in the CA1 area of the hippocampus, activates ERK1/2 in this region. Pharmacological inhibition of MEK, using PD98059 or SL327 before tetanization, blocked the induction of LTP in this area *(20,44).* Similarly, in an in vivo model of LTP in the dentate gyrus, ERK1/2 activation is also crucial for LTP induction and maintenance *(45,46).* This ERK-dependent LTP is controlled by both muscarinic and NMDARs *(46).* Furthermore, the ERK1/2 cascade seems to be required for multiple forms of synaptic plasticity in the rat hippocampus, since PD98059 and/or U0126 prevent the LTP induced by very high-frequency stimulation (200 Hz) or by the K^+-channel blocker, tetraethylammonium, two described models of NMDAR-independent LTP *(47,48).* Ras-GRF is highly expressed in the CA1 region of the hippocampus, as well as in the amygdala. However, in Ras-GRF-deficient mice, NMDAR-dependent LTP reveals no impairment in hippocampal synaptic plasticity. In contrast, LTP is severely impaired in the lateral-basolateral amygdala of these mice *(28).* Furthermore, bath application of U0126 to amygdala slices impaired LTP in the lateral amygdala, without affecting synaptic transmission *(27).* In this latter structure, LTP is highly dependent on muscarinic acetylcholinergic, and not NMDA, receptor activation *(28),* suggesting that Ras-GRF can be involved in synaptic plasticity models requiring GPCRs activation, rather than calcium influx mediated by NMDARs. However, the authors cannot exclude the possibility that mobilization of intracellular Ca^{2+} stores is not involved in this process, since muscarinic receptors are positively coupled to PLC. Finally, ERK-dependent LTP, induced in the insular cortex by stimulation of the basolateral amygdala, requires both functional NMDARs and modulation by muscarinic acetylcholine receptors *(49).*

Thus, multiple extracellular stimuli, and thereby different intracellular second messengers, can account for ERK activation in discrete brain area (*see* below). The role of ERKs in synaptic plasticity is conserved during evolution, because inhibiting their function results in blockade of long-term facilitation in *Aplysia*. However, although ERK activation is required, in rodents, for both induction and maintain of LTP, it plays a critical role in late, but not early, long-term facilitation in aplysia *(50),* which

suggests that ERKs may differently regulate intracellular substrates in these species. Critical issues now are, first, to establish upstream events implicated in neurotransmitter-induced ERKs activation, and second, to understand how ERKs activation may underlie long-term neuronal adaptive responses.

Upstream Events Implicated in Neuronal ERK Activation

The two major ERK isoforms, ERK1 and ERK2, are highly expressed in the adult CNS, in mature neurons *(14,51).* They are activated by multiple neurotransmitters, and thereby respond to various intracellular second messengers, mainly Ca^{2+} and cyclic adenosine monophosphate (cAMP), which are known to play a key role in model systems of synaptic plasticity in rodents.

Although intracellular mechanisms involved in activity-dependent ERK phosphorylation in neurons have already been extensively reviewed elsewhere *(3,13),* the authors here summarize major upstream events critically involved in ERK-dependent synaptic plasticity and/or memory formation (Fig. 1).

Briefly, increases in intracellular Ca^{2+} levels can activate the Ras/ERK pathway via nonreceptor tyrosine kinases, such as the proline-rich tyrosine kinase 2 *(52)* or the focal adhesion kinase ($pp125^{FAK}$) *(53).* Autophosphorylation of $pp125^{FAK}$ on Tyr397 occurs after Ca^{2+} increases, and allows its interaction with Src *(54),* which in turn can phosphorylate $pp125^{FAK}$ on Tyr925, leading to its recruitment at the plasma membrane, and to the subsequent recruitment of Grb2 and Sos, two well-known activators of Ras *(55).* Autophosphorylated $pp125^{FAK}$ can also interact with $pp59^{FYN}$ *(56),* a Src family protein involved in hippocampal LTP and spatial learning, as evidenced from mice bearing mutations in the $pp59^{FYN}$ gene *(57).*

Several brain-specific guanine nucleotide exchange factors (GEFs), which allow activation of small G proteins of the Ras family, may also provide a molecular basis for neurotransmitter-induced ERKs activation. These GEFs are sensitive to increases of intracellular Ca^{2+} and/or diacylglycerol (DAG) as well as to cAMP levels. They allow activation of Ras-related proteins, such as Ras itself, or Rapl, in response to VSCCs, ionotropic NMDARs, and GPCRs. Among these GEFs, one of the best-characterized, the (Ras-GRF), is directly activated by Ca^{2+}/camodulin, in response to intracellular Ca^{2+} increases *(58).* Thus, mobilization of intracellular Ca^{2+} by GPCRs could account for activation of Ras-GRF in the amygdala. Other GEFs (CalDAG-GEF I and CalDAG-GEFII) can activate Rap1 and Ras, respectively, in response to elevation of (DAG) and Ca^{2+} *(59,60).* Those authors also identified a cAMP-GEF that is responsible for Rap-1-dependent ERK activation following increases of intracellular cAMP *(61,62).* However, there is no evidence so far for a role of these GEFs in ERK-induced neuronal adaptation.

Proteins that negatively control Ras activity can also be involved in synaptic plasticity and/or learning and memory. In this way, synGAP, a protein highly enriched at excitatory synapses in the hippocampus, inhibits ERK activity by stimulating a Ras-GTPase activity *(63).* Indeed, Chen et al. showed that ERK activation, induced in the hippocampus by increases of Ca^{2+} influx through NMDARs, was tightly linked to inactivation of synGAP resulting from phosphorylation by activated CaMKII *(64).* Given the key role of ERK activation in hippocampal LTP in vitro and in vivo, synGAP may play an important role in synaptic plasticity. Evidence for a role of Ras-GTPase in memory formation is also supported by behavioral data from mice deficient for NF1 *(24)* (*see* Table 1).

Finally, one must keep in mind that ERK cascade may represent a convergent point for other intracellular signaling pathways, such as CaMKII, PKA, or PKC *(3,65,66).* The well-documented role of these kinases in synaptic plasticity and memory formation could be

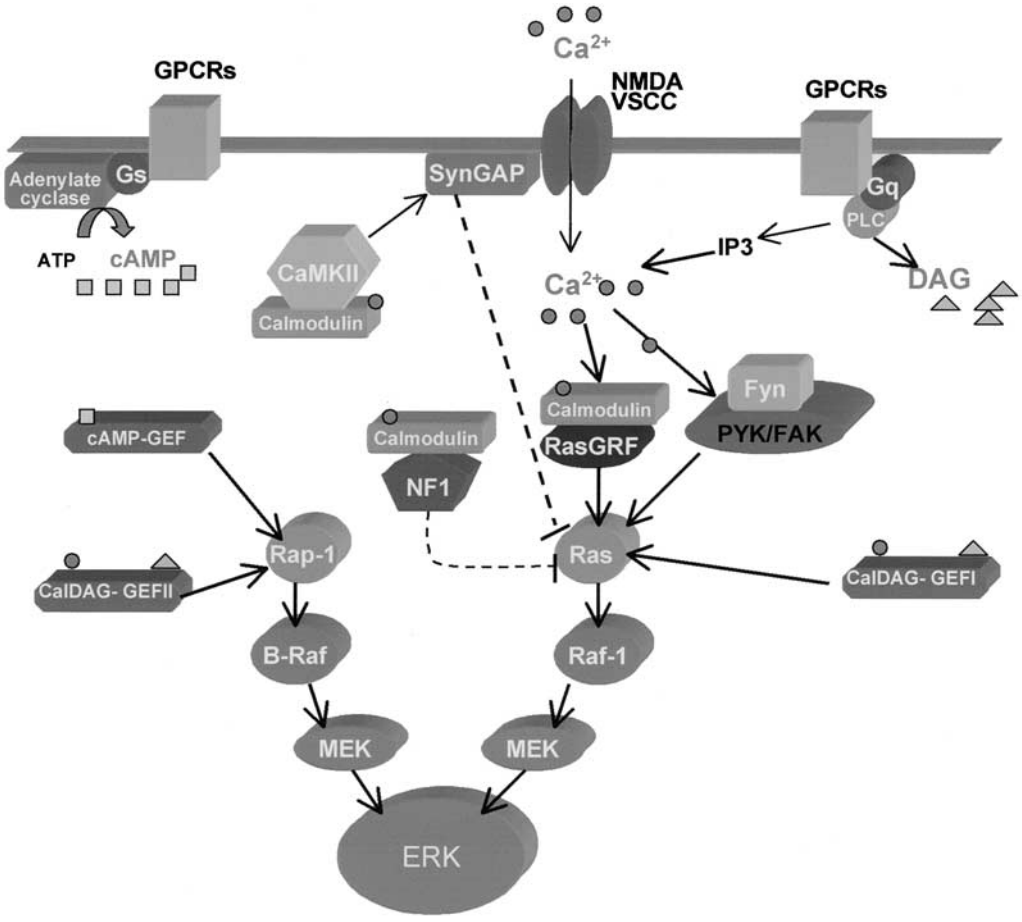

Fig. 1. Upstream events involved in ERK signaling in neuronal cells. Intracellular Ca^{2+} elevation mediated by VSCC, NMDA or Gq-coupled receptors, can activate Ras and/or Rap1 via multiple pathways. One implicates the nonreceptor tyrosine kinases, PYK or FAK, after binding of Src family proteins, such as Fyn. The neuronal specific GEF Ras-GRF responds to direct binding of calmodulin, and Ca1DAG-GEF1 is activated by Ca^{2+} and DAG. Activation of the Ras-GTPases, SynGAP by CaMKII and NF1 by calmodulin, leads to Ras desinhibition. Intracellular cAMP elevation, mediated by Gs-coupled receptors, leads to Rap1-dependent activation of ERKs via cAMP-GEF and Ca1DAG-GEFII.

mediated, at least in part, by the recruitment of the ERK cascade.

Substrates of ERKs that May Underlie Long-Term Neuronal Adaptive Responses

The fact that interfering with ERK activity blocks LTP, more rapidly than one would expect for a cascade involved in the protein-synthesis phase of LTP, suggests that ERK1/2 is involved in phophorylation of pre-existing cytoplasmic proteins. In their nonphosphorylated state, ERK1/2 are mainly localized in somatic and dendritic compartments of neurons *(16,51)*. Upon activation, they target numerous substrates, which may be mem-

brane-associated proteins, such as EGF receptors, phospholipase A2, cytoskeletal proteins, including microtubule associated proteins, and neurofilaments *(3).* Phosphorylation of these cytoskeletal proteins by ERKs could arbitrate morphological changes that underlie plastic properties of neurons. Another strong candidate is synapsin I, a presynaptic protein, whose phosphorylation by ERKs has been reported to facilitate glutamate-evoked release *(67).* This may account for the acute facilitating role of ERKs in synaptic efficacy.

Blocking Ras/ERK activation interferes poorly with STM formation, or with acute behavioral responses to cocaine. By contrast, LTM formation *(19,25,27,28),* as well chronic behavioral adaptation to cocaine *(17,18),* are strongly dependent on Ras/ERK signaling. Altogether, these data strongly indicate that ERK-controlled gene expression and new synthesis proteins are important steps for the establishment of these behaviors. Upon their activation, ERKs translocate to the nucleus *(68),* where they play a major role in the control of gene expression. In neuronal models, in vitro or in vivo, immunocytochemical data, utilizing a specific anti-active ERK antibody, clearly show a nuclear localization of activated ERKs. For example, LTF in Aplysia *(50),* in vivo electrical stimulation of the corticostriatal pathway *(69,70),* LTP in the hippocampus *(45)* or acute cocaine administration *(18)* are all characterized by nuclear localization of activated ERKs. Blocking ERK activation interferes with immediate-early gene (IEG) (c-*fos, zif268, MKP-1* mRNA) induction, by corticostriatal stimulation *(70).* Similarly, *zif268* mRNA expression, induced in LTP in the dentate gyrus in vivo *(45)* or striatal induction of c-Fos proteins in response to cocaine *(18),* are blocked by a MEK inhibitor. When phosphorylation of ERKs remains cytoplasmic, e.g., in the case of partial inhibition by SL327, IEG mRNA induction is impaired *(45).* Thus, nuclear translocation of activated ERKs appears necessary for triggering transcriptional regulation of IEGs that is required for long-term synaptic plasticity *(71–73).*

Substrates of ERKs Involved in Gene Regulation

Molecular mechanisms underlying nuclear translocation of activated ERKs seem to be complex, and are only partly understood. Indeed, ERKs do not contain any nuclear localization signal, which suggests that they need a partner protein to facilitate their accumulation in the nucleus. Under basal conditions, ERKs are maintained in the cytoplasm through interaction with their upstream kinases MEKs. When ERKs are phosphorylated, they dissociate from MEKs *(74).* This may participate to nuclear translocation of ERKs after formation of homodimers or heterodimers of ERK/activated ERK *(75).* Alternatively, other PKs, such as the cytoplasmic CREB-kinase ribosomal S6 kinase 2 (RSK2), which possesses a nuclear localization signal *(68),* are candidate partner proteins to facilitate nuclear translocation of activated ERKs *(76).*

In the nuclear compartment, activated ERKs directly target numerous transcription factors, such as ternary complex factor (TCF), signal transducer and activator of transcription (STAT), c-Myc, or c-Jun *(3).* In the adult CNS, ERKs have been shown to control the phosphorylation state of the TCF/Elk-1 in various model systems of IEG induction *(18,45,70,77).* Elk-1 represents the first and best-identified TCF *(78),* and belongs to the ETS protein family, which also contains two other members: Sap1a and Net/Sap2/ERP *(79,80).* These proteins are constitutively bound as a ternary complex composed of a dimer of serum responsive factor (SRF) and one molecule of TCF at the SRE (serum responsive element) *(81,82).* This 20-bp sequence is located in the promoter of several IEGs (such as c-*fos, zif268, Nur77, JunB*) *(83,84).* Phosphorylation of TCFs on specific residues (principally Ser383 and Ser389 for Elk-1), by activated ERKs, seems to be both necessary and sufficient for SRE-driven transcriptional regulation *(85–89).*

ERKs also exerts, via RSK2, an indirect control on CREB *(90),* SRF *(91),* c-Fos, and Nur77

(92) phosphorylation. In pheochromocytoma 12 cells *(93),* as well as neuronal cells, ERK controls CREB phosphorylation *(45,70)* or CRE-driven gene expression via RSK2 *(76,94).* RSK2 can also control Elk-1-driven gene activation via a still-unidentified mechanism *(95).* The gene encoding RSK2 is mutated in the Coffin-Lowry syndrome in human, a disease characterized by mental retardation *(96,97).* Thus, by affecting the ability of Elk-1 to activate transcription, and by directly targeting CREB on Ser133, this ERK-activated kinase could play a key role in genetic programs that are necessary for memory formation.

ERKs also directly target the CREB-binding protein (CBP), a cofactor transactivator playing a key role in the transcriptional control of gene expression *(98,99).* CBP acts as a molecular bridge between transcription factors, such as CREB *(100),* Elk-1 *(101),* Sap1a *(102),* STAT *(103),* c-Jun *(104),* or c-Fos *(105),* and proteins belonging to the basal transcriptional machinery, such as the RNA polymerase II *(106),* RNA helicase A *(107),* or Tata box-binding protein via TFIIB *(108).*

ERK: Integrator of Activity-Dependent Gene Regulation?

By regulating the phosphorylation state of numerous transcription factors, as well as that of CBP, the Ras/ERK pathway appears to an integrator of activity-dependent gene regulation involved in long-term neuronal adaptation.

A good example of the integrating role of ERK cascade in gene induction comes from studies aimed at analyzing intracellular events governing regulation of the c-*fos* proto-oncogene, perhaps the best-studied IEG. Analysis of the c-*fos* promoter has provided a structural framework useful for studying how the activation of intracellular signaling cascades leading to changes in gene expression. This IEG is activated transcriptionally by a plethora of extracellular stimuli (growth factors, cytokines, electrical or pharmacological stimuli, environmental stress), thus indicating that its promoter is a convergence point for multiple intracellular signaling network *(109).* Two DNA regulatory sequences located in this promoter, the CRE and the SRE sites, play a key role in c-*fos* transactivation in neuronal cells in vitro *(110,111),* and increasing evidence now indicates that synergistic activation of these sites is necessary for full activation of c-*fos.* The first, an elegant in vivo study from Robertson et al. *(112),* showed that mutating either of these DNA sequences strongly impaired Ca^{2+}-driven induction of a c-*fos* reporter gene in transgenic mice. Similarly, transfection studies imply a role for these two elements in neurotrophin activation of IEGs *(113),* and show that multiple Ca^{2+}-dependent pathways target them *(114).* Most in vivo studies in the CNS, aimed at analyzing intracellular mechanisms leading to c-*fos* induction in response to glutamate, show a combined activation of Elk-1 and CREB *(70,77).* In contrast, DA-induced c-*fos* transcription involves both ERK-dependent and -independent pathways *(18).* Thus, depending on the extracellular stimuli, and thereby second messengers recruited by these stimuli, i.e., Ca^{2+} in the case of glutamate or cAMP for DA, ERK can control both CRE and SRE sites or the SRE site alone. Furthermore, c-*fos* gene induction is also dependent on CBP *(115,116),* which may interact with components of the basal transcription machinery, together with the multiprotein complexes bound to the CRE or the SRE sites *(117).* The interaction of CBP with transcription factors bound to these sites, i.e., CREB and Elk-1, respectively, is independent on their phosphorylation, all proteins being in place prior to the stimulation. Transactivation of c-*fos* could result from phosphorylation-dependent conformational changes of these proteins. Since, among these proteins, CREB, Elk-1, and CBP all are potent targets of ERKs, at least upon Ca^{2+} signaling, this could provide a molecular basis for explaining the critical role exerted by ERK-signaling on c-*fos* expression (Fig. 2).

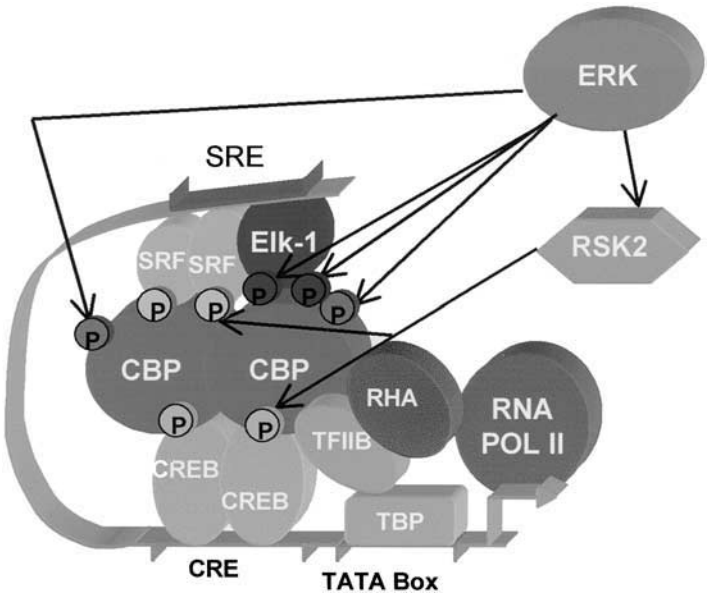

Fig. 2. ERK: an integrator of activity-dependent at the c-fos promoter. Direct nuclear targets of ERKs are Elk-1, bound at the SRE, together with two molecules of SRF (serum response factor) and the adaptor molecule, CBP. Phosphorylation of Elk-1 at Ser383 and Ser389, by activated ERKs, strongly potentiates its binding to SRE, and thereby c-fos transcription. Furthermore, ERKs phosphorylate the cytoplasmic CREB kinase, RSK2, which in turn translocates to the nucleus and controls CREB phosphorylation. All these proteins (CREB, Elk-1, and CBP) are in place on the c-*fos* promoter, prior to stimulation. Their combined phosphorylation could produce conformational changes necessary for activating the transcriptional complex bound to the RNA, polII.

Conclusion

Altogether, these data strongly support a key role for ERK in a rapid and transient burst of gene expression that is necessary for long-term neuronal adaptation. Most ERK-controlled IEGs identified so far encode transcription factors (c-*fos* and *zif268*), which regulate in turn a second wave of gene expression coding for proteins possibly involved in synaptic efficacy. For example, by interacting with other members of the AP1 family (c-Jun, JunB), c-Fos could control tyrosine hydroxylase *(31,118,119),* the rate-limiting enzyme in DA synthesis. *zif268* is known to control expression of the NMDAR subunit, NR1 *(120),* and could thereby contribute to reinforcing synaptic efficacy necessary for LTP.

Besides transcription factors, ERKs also control expression of MAP kinase phosphatase 1, a gene containing a CRE site in its promoter *(70).* This gene encodes a protein involved in the negative feedback control of ERK activation *(121).* Thus, it is reasonable to speculate that, given the potential role of ERK in the control of CREB phosphorylation, other genes carrying CRE sites could be under its control. For example, in Aplysia, LTF-induced transcriptional regulation of ubiquitin hydrolase (a degradation enzyme for PKA regulatory subunits) *(122),* is controlled by CRE sites. This results in constitutive activation of catalytic subunits, therefore reinforcing the cAMP-induced role in long-term facilitation. CREB is able to directly regulate the transcription of the brain-derived neurotrophic factor gene *(123–125),* which is

known to facilitate hippocampal LTP *(126).* Thus, ERK signaling to CREB could participate in the well-established role of this transcription factor in memory formation and learning *(127).*

Increasing evidence now implicates the transcription factor, Elk-1, a direct target of ERK in neuronal cells *(66),* in various model systems of long-term adaptation, such as conditioning to an aversive taste *(25),* one-trial avoidance *(30),* LTP *(45),* or cocaine administration *(18).* By controlling expression of genes having SRE sites in their promoter (such as c-*fos, zif268, nurr77,* or *junB*), this transcription factor is a strong candidate for ERK-induced long-term neuronal adaptation. Further experiments, with genetically modified mice for Elk-1, will be required to further analyze genes targeted by this transcription factor, its specific role in synaptic plasticity, and LTM formation.

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Abbreviations

AT, after training; BT, before training; CamK, calcium/calmodulin-dependent kinase; CBP, CREB-binding protein; CRE, cAMP and calcium-responsive element; CREB, cAMP and calcium-responsive element binding protein; ERK, extracellular signal-regulated kinase; GEF, guanine nucleotide exchange factor; GPCR, G-protein-coupled receptor; IEG, immediate-early gene; JNK, c-Jun N-terminal kinase; LTM, long-term memory; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; PKA, cAMP-dependant kinase; Ras-GRF, Ras guanine-nucleotide releasing factor; RSK2, Ribosomal S6 kinase 2; SRE, serum response element; SRF, serum response factor; STM, short-term memory; TCF, ternary complex factor; VSCC, voltage-sensitive calcium channel.

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