Genetics of Childhood Disorders: LII. Learning and Memory, Part 5: Human Cognitive Disorders and the ras/ERK/CREB Pathway

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Over the last few columns we reviewed how different forms of learning and memory occur in specific regions of the CNS. Spatial learning involves long-term synaptic changes in the hippocampus, while fear conditioning involves similar long-term synaptic changes in the amygdala. Key molecular players in the processes governing long-term synaptic modifications are members of the mitogen-activated protein kinase (MAPK) family. Within this family, the *extracellular signal–regulated kinases*

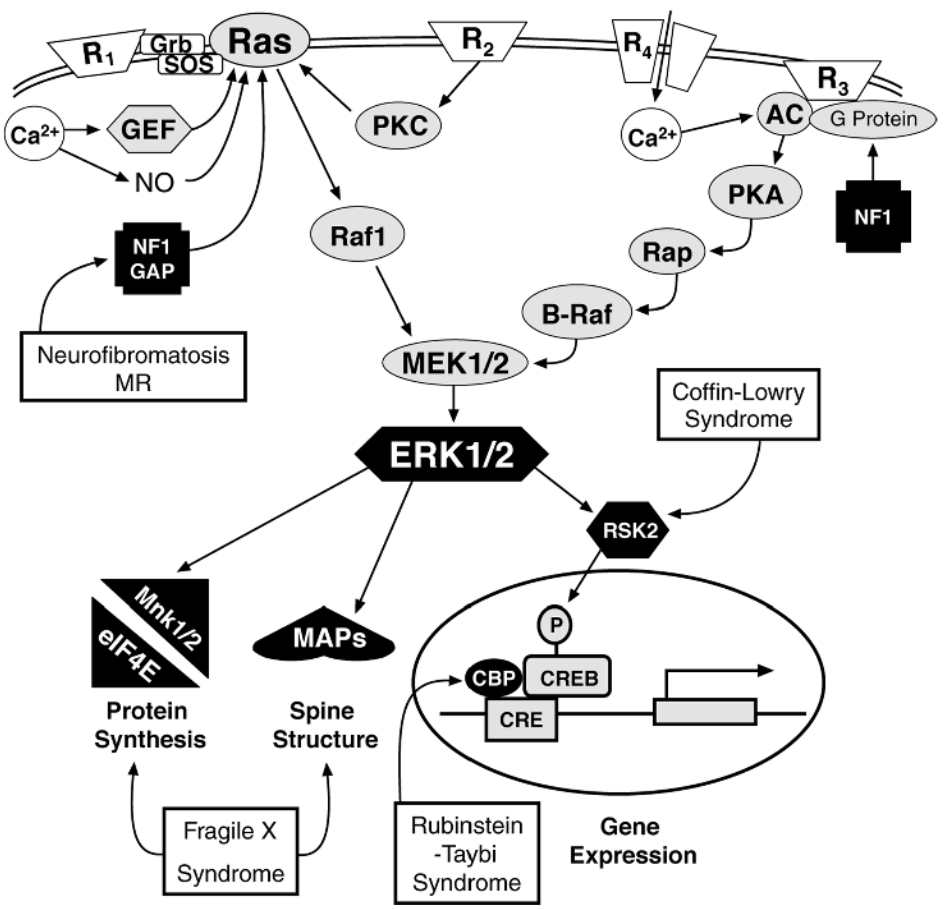

Fig. 1 The ERK MAPK cascade affected in human mental retardation syndromes. The mitogen-activated protein kinase (MAPK) family includes two important members that are involved in many different forms of learning and memory. These proteins are termed extracellular signal–regulated kinases (ERKs) to reflect the fact that they were initially characterized as kinases that became activated after growth factors bound to specific receptors at the outer surface of a cell's membrane. Upstream regulators and selected potential downstream targets of the ERK MAPK cascade are shown, along with known sites of derangement in human mental retardation syndromes. One current model is that MAPK plays multiple roles in memory formation: modulating the induction of lasting synaptic changes through regulating voltage-dependent potassium channels, and triggering long-lasting changes through regulating gene expression via CREB phosphorylation. Other possible sites of action are regulating local protein synthesis, regulating cytoskeletal proteins, and regulating other ion channels such as the AMPA subtype of glutamate receptor.

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(ERKs) are required in many different forms of learning in many species. Thus activation of ERK-1 and ERK-2 is part of the conserved core signal transduction cascade necessary for mammalian learning and memory. Activation of these kinases leads to the phosphorylation of downstream target proteins.

What are the molecular and cellular targets of this cascade? Given that ERKs play an important role in integrating signals in the hippocampus and other memory-related areas of the CNS, what is the read-out of the ERK signal? What follows is a brief description of several of the key targets of ERKs: transcription factors, the protein synthesis machinery, the cytoskeleton, and cell surface glutamate receptors.

Transcription Factors. The ERK cascade regulates gene expression. This was suggested by the observation that ERK1/2 are rapidly translocated into the nucleus. We now know that several transcription factors are phosphorylated by ERK1/2, including CREB, Elk-1, and c-Myc. Phosphorylation of these transcription factors leads to enhanced *transcriptional activity*. Prominent among the transcription factors regulated by the ERKs is Elk-1. When phosphorylated at multiple sites by ERK1/2, Elk-1 cooperates with another transcription factor, serum response factor, to drive transcription of serum response element (SRE)-controlled genes.

The cAMP responsive element binding protein (CREB) is another transcription factor that has been extensively studied in relation to ERK's role in regulating gene expression. CREB activates gene transcription when phosphorylated at a serine at amino acid position 133 (ser133). The ERK cascade is coupled to CREB phosphorylation through the activation of another family of kinases, called *ribosomal S6 kinase 2 (RSK2)*. Ser133 of CREB is not a substrate for ERK. Instead, ERK affects CREB indirectly by activating RSK2. Phosphorylation of CREB at ser133 by PKA, RSK2, or other kinases recruits the CREB binding protein, CBP, to the initiator complex and thereby promotes the transcription of target genes. Many genes are activated by CREB, including other transcription factors such as c-fos. In this way, CREB signaling can indirectly activate an expanded range of genes. It is interesting that the cAMP pathway uses the MAPK cascade as an obligatory intermediate in regulating CREB phosphorylation in hippocampal area CA1, an area of the brain critical for learning and memory. As we will see below, deregulation of CREB signaling leads to several mental retardation syndromes.

Protein Synthesis. A second ERK1/2 target is the machinery that regulates protein synthesis. Protein synthesis begins with the recognition of an mRNA by the ribosomal complex, which allows the initiation of peptide chain elongation starting from the 5′ end of the message. The initiation of translational mechanisms involves the eukaryotic translation initiation factor 4e (eIF4e). Activated eIF4e associates with a number of coactivating proteins, and this complex recruits the ribosome to the mRNA, which initiates the process of scanning for the site to begin translation (at the AUG start codon). eIF4e activation is regulated by phosphorylation at one major site, ser209. The kinase likely to mediate this phosphorylation is MAPK-interacting-kinase 1 (MNK1). As the name suggests, MNK1 is regulated by ERK through direct phosphorylation and activation.

Another ERK target that may transduce a signal in the protein synthesis machinery is RSK2, which we mentioned in the context of CREB phosphorylation. As described above, ERK directly phosphorylates and activates RSK2, which acts upon the ribosome complex. While the role of RSK2 in regulating protein synthesis is not clear, both it and its target, glycogen synthase kinase 3β (GSK-3β), are thought to regulate protein synthesis through the phosphorylation of ribosome-associated initiation factors. One specific candidate in this context is eIF2B. Although the mechanisms by which the ERK pathway regulates protein synthesis are still being defined, it is appealing to hypothesize a role for this cascade in regulating neuronal protein synthesis. New protein synthesis is required during the synaptic modifications necessary for long-term synaptic plasticity.

Cytoskeletal Proteins. A third target of the MAPK family are cytoskeletal proteins. The phosphorylation of these proteins leads to structural changes within a cell. MAPK is an acronym for mitogen-activated protein kinase, but this was not the original definition. "MAPK" originally stood for microtubule-associated protein kinase, which phosphorylates MAPs (microtubule-associated proteins). ERK MAPK was originally identified as a kinase that phosphorylated cytoskeleton-associated proteins, and was redefined as the mitogen-activated protein kinase when its role in cellular regulation became clear. Historically, the MAPK acronym has meant two different things but it has always referred to the same kinase.

MAPs, the ERK substrates that led to the original name, regulate microtubule structure and stability. Their effects on microtubules are in turn regulated by phosphorylation. ERKs and other kinases acting upon the MAPs regulate cytoskeletal assembly and turnover, and by this mechanism regulate cell morphology. The cytoskeleton was the first function attributed to the ERK signal transduction cascade. Changes in neuronal morphology constitute one mechanism by which neurons adapt their synaptic connections in response to repeated synaptic activity, a key aspect of the molecular basis of learning.

Postsynaptic Glutamate Receptors. The last ERK target to be discussed in this column is another component of the molecular mechanism of learning and memory, postsynaptic glutamate receptors. There are several potential mechanisms by which neuronal signaling may be strengthened. One possibility is to increase the amount of neurotransmitter released at the synapse. This does occur over the short term; it does not require protein synthesis and is governed primarily by post-translational modification of proteins already present within the synapse. For example, the level of phosphorylation of the

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proteins within the presynaptic terminal determines the number of synaptic vesicles that fuse with the membrane. This, in turn, regulates the amount of neurotransmitter released with each action potential.

A second potential mechanism for strengthening neuronal signals would be to increase the number of synapses. This is a means of storing long-term memories. ERKs are required for this process because they phosphorylate the transcription factors that lead to new protein synthesis and structural changes at the synapse.

Permanent increases in synaptic strength might result from increases in the number of *postsynaptic glutamate receptors*. One aspect of this phenomenon is fairly well understood—increases in the postsynaptic "AMPA" subtype of glutamate receptors. Augmenting postsynaptic AMPA receptor function is accomplished, in part, by increasing the steady-state level of membrane AMPA receptor protein. This is done by regulating AMPA receptor trafficking and stabilization. In other words, the number of AMPA receptors in the membrane is not constant over time, but changes quite rapidly depending on the level of electrical activity at the synapse. AMPA receptors can be inserted into previously *silent synapses,* increasing the strength of connections between two neurons in an essentially all-or-none fashion. It has been proposed that this mechanism contributes as well to long-term potentiation (LTP), especially in early developmental stages. A variety of sophisticated studies from Robert Malinow's laboratory show that increased AMPA receptor trafficking into dendritic spines and active synapses can be encouraged by placing active calcium/calmodulin-dependent protein kinase II (CaMKII) into pyramidal neurons. Similar processes have been observed with LTP induction in cultured neurons in vitro. These model systems have been used successfully to investigate the mechanisms underlying the insertion and removal of AMPA receptors.

While the mechanisms of AMPA receptor trafficking are quite complex, one component is known to be dependent on ERK activity. Specifically, new work from Malinow's laboratory has shown that ERK inhibition blocks CaMKII-triggered AMPA receptor expression on the neuronal surface and disrupts LTP. It appears that ERK may participate in synaptic plasticity by regulating the AMPA receptor insertion mechanism triggered by increasing synaptic strength.

A number of investigators have recently capitalized on the opportunities presented by genetic engineering in mice to investigate naturally occurring, genetically based, human mental retardation syndromes. The approach uses genetic mutations in humans that are known to cause mental retardation and learning disorders to make knock-out and transgenic mouse models of the human disorders. These models help generate insights into the molecular and cellular mechanisms underlying learning and memory defects in humans. Although this approach is at very early stages, several studies have already begun to point to the ras/ERK cascade and its associated upstream regulators and downstream targets (Fig. 1).

An elegant example comes from studies done by Alcino Silva's laboratory at UCLA, which focused on human *neurofibromatosis-associated mental retardation*. Neurofibromatosis is an autosomal dominant disease that exhibits a variety of clinical features, principally benign tumors of neural origin. Other potential characteristics include café au lait spots and skeletal malformation. The gene mutation that causes human neurofibromatosis is the *neurofibromatosis type 1 oncogene, NF1*. Mutations in *NF1* cause mental retardation in about 50% of cases. There is a great deal of variability in presentation of the mental retardation phenotype as a consequence of the heterogeneity in the types of *NF1* gene mutations (e. g., different point mutations or deletions). Although neurofibromatosis was initially identified and named for the neurofibroma tumor phenotype, the genetic mutation may cause mental retardation regardless of the presence or absence of tumors.

The product of the *NF1* gene is neurofibromin, a molecule that has the capacity to regulate several intracellular processes including the ERK cascade. A sophisticated study by Alcino Silva and colleagues used knock-out mouse technology to identify a specific domain in the NF1 protein that is critical for learning. Mice lacking this domain exhibited learning problems but no apparent developmental abnormalities or predisposition for tumors. This suggested that the protein domain is selectively involved in the mental retardation phenotype. Further studies demonstrated that this stretch of amino acids regulates the GTPase activating protein (GAP) domain of NF1, a domain that regulates interaction with the NF1 target ras. Ras is a low molecular weight guanine nucleotide binding protein at the cell membrane that leads to the sequential activation of several kinases and eventual activation of ERK1/2 (previous column and Fig. 1).

Silva and his colleagues hypothesized that the loss of NF1 regulation leads to ras hyperactivation. This, in turn, leads to inappropriate activation of the ERK cascade, causing the learning disorder phenotype in NF1-deficient animals. In an important paper published recently in *Nature,* Silva and colleagues reported directly testing their hypothesis. They decreased the level of active ras in defective animals in an effort to restore proper levels of ERK1/2 activity. They used a classical genetic approach, diminishing ras function by removing one of the two ras genes present on the homologous chromosomes. They also used a pharmacological inhibitor of ras activity in vivo to probe for interactions of the *NF1* gene product with the ras pathway. Their work elegantly demonstrated that the NF1 deficiency alters ras activation of ERK, and that by diminishing ras activity, they could restore more normal levels of ERK1/2 activity. This, they concluded, is the basis for the learning and synaptic plasticity deficits observed in their mouse models of human NF1-related mental retardation.

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Other recent studies have implicated the ras/ERK pathway in other human mental retardation syndromes. An ERK target in the hippocampus is the transcription factor CREB. ERK couples to CREB through the intervening kinase RSK2, which phosphorylates CREB at ser133 and thus regulates gene expression. This is important in the present context because RSK2 is the gene disrupted in human *Coffin-Lowry mental retardation syndrome*. Therefore, the same pathway implicated in studies of neurofibromatosis mental retardation, the ERK/RSK/CREB pathway, has also been implicated in studies of Coffin-Lowry syndrome. Current thinking is probably oversimplified, but it is intriguing that the same signal transduction cascade, the ras/ERK/CREB cascade, is involved in two completely different human mental retardation syndromes. This is compelling evidence for a role for this cascade in both normal human learning and human mental retardation.

Finally, a third mental retardation syndrome associated with the PKA/ERK/CREB pathway is *Rubinstein-Taybi syndrome (RTS)*. RTS patients have some facial abnormalities, broad big toes and thumbs, and mental retardation. The *RTS* gene has been mapped to chromosome 16 and identified as *CREB binding protein (CBP)*. CBP is a transcriptional coactivator with CREB that participates with phospho-CREB to regulate gene expression. One mechanism by which CBP promotes gene expression is through histone acetylation, a process that opens up and exposes the otherwise tightly bound, double-stranded DNA to the transcriptional machinery. The loss of this CBP function contributes to the development of RTS. Investigators have developed a partial knock-out mouse model in which CBP activity is lost and animals exhibit learning deficiencies.

There are two take-home messages. One is the emerging importance of the ras/ERK/CREB pathway in learning and memory. The role of this pathway in learning in animal model systems has been under investigation for some time, and its relevance in this context is now fairly clear. However, exciting new work has begun to highlight its involvement in human learning disorders as well—a nice convergence of animal and clinical studies upon a common theme of signal transduction processes in memory formation.

The second take-home message is that key discoveries concerning the basic biology of synaptic plasticity and memory formation have relied on genetically engineered mice. This trend is bound to continue as more laboratories turn to genetically engineered mouse models of human disorders to gain new insights into learning disabilities. The power of the genetic engineering approach is expected to be increasingly apparent in the context of understanding human cognitive disorders.

WEB SITES OF INTEREST

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