# Genetics of Childhood Disorders: LI. Learning and Memory, Part 4: Human Cognitive Disorders and the ras/ERK/CREB Pathway

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It is difficult to overstate the likely impact of recent advances in mammalian genetic engineering. The ability to engineer the mammalian genome will eventually rank along with splitting the atom, developing the computer, and inventing the printing press in its impact on the human condition. If taken to its limits, genetic engineering could allow us to restructure ourselves at the molecular level—a thought both ethically sobering and medically promising.

Emerging genetic engineering technologies allow us to delete, mutate, and overexpress mammalian genes in a variety of animal models. It is also possible to introduce genes from one species into another, thereby adding to its cellular milieu a new

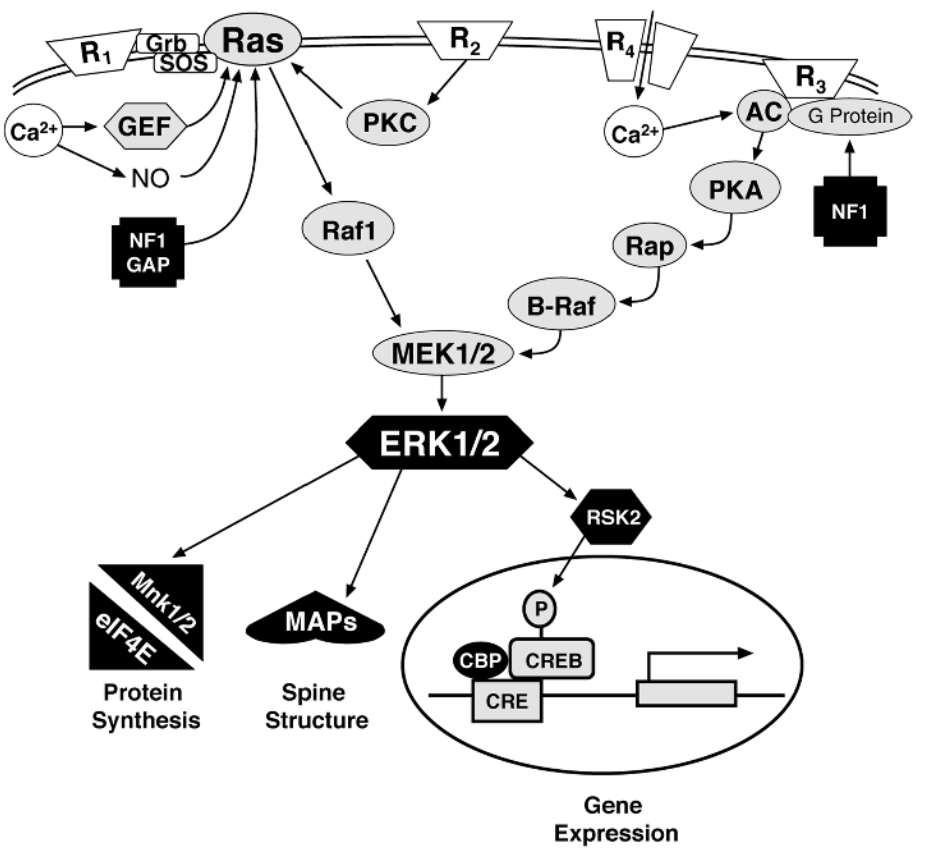


**Fig. 1** The ERK MAPK signaling pathway. The mitogen-activated protein kinase (MAPK) family includes two important members that are involved in many different forms of learning and memory. These proteins are termed extracellular-signal regulated kinases (ERKs) to reflect the fact that they were initially 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. 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.

protein not previously available to that species. This has been used to confer disease resistance from one plant to another. Plant genes can also be introduced into the mammalian genome for use as unique cell tracing markers. Developing genetic engineering technologies will soon allow us to remove a gene at different times during development: from conception up through adulthood. This will help investigators to determine the function of specific genes. The development of gene deletion technologies specific to particular tissues, organ systems, brain regions, and cell types will allow us to implement sophisticated and highly restricted gene product excisions in particular subdomains of the body.

At present, most of these technologies are being developed in mice. Genetic engineering first became a practical reality in mice largely because of their convenient size, short generation time, and long history in experiments in mammalian genetics (many varieties of mouse mutants are commercially available and the mouse genome is relatively well understood). Mouse embryonic stem cells are particularly robust when applied to *homologous gene recombination* experiments. In this process, an altered gene introduced into the mouse genome replaces the normal, unaltered variant, a first step in the production of what is commonly referred to as *knock-out mice*. For these reasons, the laboratory mouse currently represents the state of the art in genetic engineering technology.

Understanding and treating human medical disorders is an important application of these technologies. In this column and the next, we address the contribution of genetic engineering technology to our understanding of the molecular basis of human mental retardation syndromes. These advances have been based on recent in vitro studies as well as the use of genetically engineered mice. A unified model is beginning to emerge that helps explain the complexities of the molecular signal transduction pathways that underlie mammalian learning and memory. This, by extension, is improving our understanding of cognitive disorders related to these processes. The present column describes the major signaling pathways involved in normal learning and memory, and the next column reviews disruptions in these pathways and their contribution to abnormal cognitive development.

The most sophisticated approaches to genetic engineering have been developed in laboratories focused on understanding the molecular basis of learning and memory. Two Nobel laureates, Susumu Tonegawa and Eric Kandel, along with their students and colleagues, have pioneered the use of genetically engineered mouse lines to investigate specific modification of the mouse genome. These models of cognition are advantageously positioned to lead us to further insights into the molecular underpinnings of higher-order CNS function. These investigators have discovered that learning activates specific signaling pathways that lead to the phosphorylation of downstream target proteins associated with the formation of memory. First we review how phosphorylation leads to protein activation, and then we turn to the molecular pathways and proteins involved in learning and memory.

After a cell assembles a specific protein from a string of amino acids (under the direction of a specific gene), many proteins undergo additional changes before they can function. This is called *posttranslational modification*. One of the most common posttranslational modifications is the addition of one or more phosphate groups. When these bulky and highly charged moieties are added, the protein undergoes a change in structure: a conformation change. For example, the addition of a phosphate group may cause a conformation change that brings a previously hidden amino acid sequence from the central core of a protein to the surface, where it can interact with target proteins. Thus the addition or removal of phosphate groups can alter a protein's enzymatic activity or its ability to interact with other proteins. This process is common to many organisms and appears to have been an early event in the evolution of eukaryotic cells. Several families of enzymes have evolved over time that either add or remove phosphate groups. The class of enzymes that adds phosphates are *kinases* and the enzymes that remove them are *phosphatases*.

Typically, kinases or phosphatases recognize specific amino acid sequences on target proteins called consensus sequences. Rapid phosphorylation or dephosphorylation of an amino acid occurs within (or adjacent to) the consensus sequence. Most kinases phosphorylate their substrates on either serine or threonine and less often on the amino acid tyrosine. For this reason, kinases are called either serine/threonine kinases or tyrosine kinases. Similarly, phosphatases are divided into either serine/threonine phosphatases or tyrosine phosphatases.

Kinases and phosphatases themselves may be regulated by phosphorylation. As with other proteins, phosphorylation of specific amino acid residues within these enzymes leads to a conformational change that changes their enzymatic activity. For example, the arrival of a chemical signal at the outer membrane of a cell often leads to the phosphorylation of a kinase on the inner side of the membrane. Once phosphorylated, the activated kinase phosphorylates another kinase, which targets its own set of proteins, potentially additional kinases. In this way, the original signal at the outer cell membrane initiates an intracellular cascade that amplifies the initial signal many times, i.e., many more of the final molecules in the pathway are activated than would be without such amplification. An important example of this type of signal amplification is the *mitogen-activated protein kinases (MAPK)* in neurons involved in learning and memory.

Kinases and phosphatases are involved in the biological functions of most cells of the body, including the CNS. The birth of neurons, their migration to specific target regions, the growth of axons and dendritic arbors, and the formation of synaptic connections are all governed by distinct phosphorylation path-

ways. Signals generated at the surface of a neuron find their way through the cell membrane and may either activate a cytoplasmic protein (such as an enzyme) or lead to gene transcription in the nucleus. The process of turning an extracellular signal into a intracellular signal is called signal transduction. This process may be initiated by an action potential, neurotransmitters, growth factors, or other chemicals that bind to specific receptors. When the end result of such a pathway is the phosphorylation of specific subsets of transcription factors, specific genes are transcribed. The protein products of the genes being expressed are those needed for the cell to function properly at that moment. In this way, different signal transduction cascades may instruct progenitor cells to divide or differentiate, or instruct a mature neuron to strengthen particular synapses through synaptic modifications that accompany aspects of learning and memory.

One central player in an emerging model of learning and memory is a signal transduction pathway known as the ERK MAPK pathway (Fig. 1). The mitogen-activated protein kinase (MAPK) cascades, as the name implies, are critical for the regulation of cell division and differentiation during nervous system development. The extracellular-signal regulated kinase (ERK) cascade is the prototypical MAPK pathway. The ERK cascade is representative of a family of signaling molecules that share a specific motif, three serially linked kinases regulating each other by sequential phosphorylation. The two ERK MAPK isoforms, named for their molecular weights as p44 MAPK and p42 MAPK, show a striking similarity to one another at the amino acid level and are referred to as ERK-1 and ERK-2 or ERK1/2.

The ERKs are abundantly expressed in neurons in the mature CNS, raising the question of why the prototype molecular regulators of cell division and differentiation are present in these nondividing, terminally differentiated neurons. One theory that has emerged is that the ERK signaling system has been co-opted in mature neurons to function in the synaptic plasticity that is required for memory. Similar to their role in nonneuronal cells, these molecules serve as biochemical signal integrators to coordinate responses to extracellular signals. We will highlight a few of the essential details of how the MAPK are regulated.

The MAPK were introduced previously when we discussed how growth factors signal to cells. When nerve growth factor is released from a neuron, it diffuses across the synaptic cleft to bind to a specific receptor on an adjacent neuron. Growth factor binding induces a conformational change in the receptor that activates the intracellular portion of the receptor. This type of receptor, designated as R1 in the accompanying figure, is a tyrosine kinase receptor. When growth factor binds to a tyrosine kinase receptor, several tyrosine residues on the intracellular portion of the receptor become phosphorylated.

The phosphorylation on tyrosine residues has an important effect. These phosphorylated residues act like a magnet, pulling several proteins out of the cytoplasm that initiate the MAPK signaling cascade. Grb, a small adapter protein, is the first protein to join the complex. It binds to the tyrosine phosphorylated residues, but has no intrinsic enzymatic activity itself. Instead, it possesses several amino acid sequences, or *motifs,* that function only to bind to other proteins and assemble them at the membrane. One end of the Grb protein binds to phosphorylated tyrosine residues on the growth factor receptor, and another portion binds to a second protein called SOS.

SOS activates ras, a very important signaling molecule located nearby on the cell membrane. Ras belongs to a family of low-molecular-weight guanine nucleotide binding proteins. This type of protein is activated by adding phosphate groups. So far, when we've spoken of protein phosphorylation, the phosphate groups were donated by an ATP and became attached to the target protein through a covalent bond. In contrast, the ras molecule is activated by binding to GTP, a molecule with properties similar to those of ATP. GTP binding activates ras and its removal inactivates ras. The addition of the GTP is facilitated by a group of enzymes called *guanine nucleotide exchange factors;* the GTP removal is facilitated by another family of proteins called *GTPase activating proteins, or GAPs*. The next column describes how a mutation in a GAP protein, which is required to return ras to its basal level of activity, causes neurofibromatosis. Under these circumstances ras cannot release the GTP molecule and it remains in a hyperactive state.

Normally, activated ras induces rapid, sequential phosphorylation of a series of kinases, ending with phosphorylation and activation of ERK1/2. ERKs, the main subject of this column, are involved in triggering long-term synaptic changes in the mammalian CNS. The earliest studies in this area showed that ERK activation plays a role in NMDA receptor-dependent *long-term potentiation (LTP)* in hippocampal area CA1. Subsequent studies showed that ERK activation is necessary for several types of long-term synaptic changes in the CNS involved in learning and memory. These include LTP in the amygdala, dentate gyrus, and regions of the cerebral cortex. LTP is believed to be the physiological basis of many forms of learning and memory; a separate column in this series will discuss LTP in greater detail. ERK activation is not universally required for synaptic plasticity in the mammalian brain, but the majority of long-term synaptic changes appear to depend on ERK activation as a triggering event.

ERKs are involved in many forms of animal learning, which is not surprising given the widespread involvement of ERKs in CNS synaptic plasticity. Associative conditioning, nonassociative conditioning, and various forms of spatial learning are all subject to disruption by inhibiting ERK activation. Some examples include fear conditioning, spatial learning in the Morris water maze, taste learning, and conditioned taste avoidance. These disparate learning paradigms in a variety of species and several brain regions, such as the amygdala, hippocampus,

and insular cortex, suggest a highly conserved role for this mechanism in learning processes through evolution.

How did investigators learn that ERKs are involved in learning and memory? This is the subject of the next column. The question, however, brings us back to our opening discussion—the importance of genetic engineering and the use of knock-out mice in investigations of cognitive function. Identifying the downstream proteins phosphorylated by ERK-1 and ERK-2 makes it possible to construct knock-out mice lacking one or another of these proteins. The absence of various members of the ERK signaling cascade have dramatic effects on the ability of knock-out mice to learn new tasks. As we will review in the next column, some of these molecules appear to be mutated in certain human developmental disorders.

## WEB SITES OF INTEREST

http://web.mit.edu/clm/ready_faculty/tonegawa.html
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# Erratum

In the article "Insecure attachment as a risk factor for future depressive symptoms in early adolescence" by Anne Mari Sund and Lars Wichstrøm, in the December 2002 issue of this *Journal*, depressive symptoms at $T_2$ were incorrectly reported as a result of a recoding error. There was, in fact, no significant increase in the proportion of high scorers (MFQ ≥ 25) in either sex from $T_1$ to $T_2$. However, the main finding of the article, that insecure attachment is a predictor of later depressive symptom level, when corrections for possible confounding were taken into account, was not changed, although the apparent size of the effect was somewhat reduced (OR = 1.22; CI: 1.03–1.44, $p < .05$). Also, as before, Alienation turned out to be the strongest predictor of depression. Now, however, the result was not significant as before, but approached significance ($p = .07$). A corrected copy of the article may be obtained from Dr. Anne Mari Sund, Department of Neuromedicine, Medical Faculty, the Norwegian University of Science and Technology, 7489 Trondheim, Norway, or via e-mail at *Anne.M.Sund@medisin.ntnu.no.* The authors regret these errors.

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