Small G proteins are an extensive family of proteins that bind and hydrolyze GTP. They are ubiquitous inside cells, regulating a wide range of cellular processes. Recently, many studies have examined the role of small G proteins, particularly the Ras family of G proteins, in memory formation. Once thought to be primarily involved in the transduction of a variety of extracellular signals during development, it is now clear that Ras family proteins also play critical roles in molecular processing underlying neuronal and behavioral plasticity. We here review a number of recent studies that explore how the signaling of Ras family proteins contributes to memory formation. Understanding these signaling processes is of fundamental importance both from a basic scientific perspective, with the goal of providing mechanistic insights into a critical aspect of cognitive behavior, and from a clinical perspective, with the goal of providing effective therapies for a range of disorders involving cognitive impairments.

In the last few years, several lines of emerging evidence suggest that small G proteins can play critical roles in many aspects of molecular processing, contributing to the alteration of neuronal function and memory formation. Small G proteins constitute a large family of proteins that bind to and catalyze guanine nucleotides (thus, they are also named small GTPases). The activity of small G proteins is regulated by their guanine nucleotide-binding state. When binding to GTP, they are in the active conformation and are able to bind to downstream effectors, whereas binding to GDP returns them to the inactive state. Small G proteins are monomeric proteins, typically between 20–25 kDa in size. Despite their small size, they play critical roles in every aspect of cellular processes. They are membrane-associated proteins, important for converting a wide range of extracellular signals to intracellular signaling cascades, as well as for mediating the traffic of small vesicles between different intracellular compartments.

The small G protein superfamily has numerous members. In this review, we focus on the Ras family proteins. However, many other families within the small G protein superfamily, such as Rho and RhoB, have been implicated in neuronal plasticity and memory as well (Newey et al., 2005; Sampson, 2009). Major members of the Ras family include Ras (H-Ras, N-Ras, and K-Ras), Rap1 (also named Krev-1), and Rap2. ras genes were first identified as oncogenes: mutations in ras genes that result in constitutively active (ca) Ras family proteins are closely associated with tumorigenesis in humans. They play important roles in mediating cell proliferation, differentiation, and survival during development (Barbacid, 1987; Der, 2006; Konstantinopoulos et al., 2007; Maruta and Burgess, 1996). Recently, a growing body of evidence suggests that Ras family proteins are also critically engaged in memory formation. Furthermore, consistent with the fact that memory formation involves modification of synapses and the intrinsic excitability of neurons in the brain (Martin et al., 2000; Mozzachiodi and Byrne, 2010; Neves et al., 2008), a number of studies have also examined the role of Ras family proteins in neuronal plasticity. These studies reveal that signaling of Ras family proteins can modify neuronal function and structure, leading to changes in synaptic strength and neuronal firing rates.

In this review, we first briefly introduce the biochemical properties of Ras family proteins and their major related signaling cascades. We then review recent studies that suggest the importance of the signaling of Ras family proteins in memory processing, followed by discussing cellular and molecular mechanisms recruited by Ras family proteins in the service of memory formation. Our goal in this review is to highlight the critical features of this family of signaling proteins in memory processing, which in turn may suggest novel therapeutic targets for cognitive impairments in disorders related to dysfunctional small G protein signaling, such as neurofibromatosis type 1 (NF1), Noonan syndrome, Tuberous sclerosis complex, autism, and Fragile X syndrome (Denayer et al., 2008b; Krab et al., 2008; Stornetta and Zhu, 2010).

Biochemical Features of Signaling by Ras Family Proteins

On-Off Switches

As with other members of the small G protein superfamily, Ras family proteins always cycle between an active conformation (GTP binding) and an inactive conformation (GDP binding). When binding to GTP, Ras family proteins slowly hydrolyze the GTP into GDP. The GDP then dissociates from the proteins, allowing for binding to GTP, which is more abundant than GDP in the cytosol. The activity of Ras family proteins can be regulated by two families of proteins (Bernards and Settleman,
Activation of guanine exchange factors (GEFs) promotes the dissociation of GDP from Ras family proteins, which facilitates the exchange of GDP for GTP, and thus enhances the activity of Ras family proteins. In reverse fashion, activation of GTPase-activating proteins (GAPs) enhances the rate of GTP hydrolysis of Ras family proteins and reduces their activity. There is a wide array of GEFs and GAPs targeting different members of Ras family proteins (Figure 1B). We should emphasize that GEFs and GAPs do more than simply turn on and off Ras family proteins. First, the activity of each GEF or GAP is under the regulation of distinct upstream signaling elements, such as cAMP, Ca²⁺, and tyrosine kinases. Thus, each of them links specific upstream signaling elements to Ras family proteins. Second, GEFs and GAPs are large, multidomain proteins. They interact with membrane lipids or other proteins, which targets them to specific intracellular compartments. Therefore, activation of a specific GAP or GEF protein can modify the activity of nearby Ras family proteins, thereby regulating specific downstream targets in a restricted cellular compartment. Finally, a subset of GAPs and GEFs target multiple small G proteins, coordinating the activity of different small G proteins to carry out coordinated biological signaling (Fan et al., 1998; Innocenti et al., 1999; Krapivinsky et al., 2004).

**Membrane Association**

Ras family proteins are localized at the cytosolic leaflet of membranes. They are targeted to membranes by a series of modifications of the C terminus. First, the activity of each GEF or GAP is under the regulation of distinct upstream signaling elements, such as cAMP, Ca²⁺, and tyrosine kinases. Thus, each of them links specific upstream signaling elements to Ras family proteins. Second, GEFs and GAPs are large, multidomain proteins. They interact with membrane lipids or other proteins, which targets them to specific intracellular compartments. Therefore, activation of a specific GAP or GEF protein can modify the activity of nearby Ras family proteins, thereby regulating specific downstream targets in a restricted cellular compartment. Finally, a subset of GAPs and GEFs target multiple small G proteins, coordinating the activity of different small G proteins to carry out coordinated biological signaling (Fan et al., 1998; Innocenti et al., 1999; Krapivinsky et al., 2004).

**Figure 1. Biochemical Properties of the Signaling of Ras Family Proteins**

(A) The cycling of Ras family proteins between inactive and active states.

(B) The signaling properties of specific inhibitors (GAP) and activators (GEF) of Ras family proteins. SEC14: Domain in homologs of an S. cerevisiae phosphatidylinositol transfer protein, lipid-binding; PH: Pleckstrin homology domain, lipid-binding; C2: membrane targeting and Ca²⁺-binding; SH3-B: Src Homology 3-binding motif; QTRV: C-terminal amino acids binding to PSD-95; ActB: actin binding domain; PDZ: anchor transmembrane proteins to the cytoskeleton; GKBD: guanylate kinase binding domain; IQ: Ca²⁺/CaM binding domain; REM: Ras-Exchanger-motif domain, stabilizing Ras activation; DEP: Dishevelled-Egl10-Pleckstrin domain, important for membrane localization; CNB: cAMP-binding domain; RA: Ras-association domain; Rac: a member of the Rho family of small G proteins.
suggests that membrane anchoring of Ras family proteins is crucial for their signaling and cellular functions (Garcia et al., 1993; Gibbs et al., 1993; James et al., 1993; Kohl et al., 1993). Inhibitors of prenylation have been designed as therapeutic tools for blocking oncogenesis (Gibbs et al., 1994; Kohl et al., 1995; Konstantinopoulos et al., 2007; Sun et al., 1995). We will return to this point in a later section when we discuss how these inhibitors have provided insights into the engagement of Ras family proteins in memory formation and neuronal plasticity. Furthermore, very recently, a number of studies have found that Ras family proteins are not stationary, but rather, they can traffic between plasma membranes and intracellular membrane compartments (such as Golgi apparatus, ER, and endosomes), which can be induced either by palmitoylation/depalmitoylation (such as in H-Ras and N-Ras) or by phosphorylation/dephosphorylation in the polybasic C-terminal region (such as in K-Ras4B). Moreover, Ras family proteins can signal from different membrane compartments which results in distinct phenotypic outputs (Bivona et al., 2003; Chiu et al., 2002; Matallanas et al., 2006; Roy et al., 2002).

**Downstream Signaling Cascades**

The best-characterized downstream signaling cascade of Ras family proteins is the Mitogen-Activated Protein Kinase (MAPK) cascade, mainly Extracellular signal-Regulated Kinase 1/2 (ERK) of the MAPK family, which has been implicated in the formation of enduring memory, but is not required for short-term memory (Adams and Sweatt, 2002; Sharma et al., 2003) (Figure 2A). When activated, Ras family proteins directly bind to the regulatory domain of Raf, thereby exposing its catalytic domain, which is subsequently phosphorylated and dephosphorylated at multiple sites for full activation. Therefore, although not sufficient, Ras family proteins are necessary for Raf activation by (1) anchoring Raf to the membrane compartment where other cofactors are present, and (2) unfolding Raf to allow for further modifications. Ras can activate either c-Raf (also named Raf-1) or b-Raf, both of which can activate MAP Kinase or ERK Kinase (MEK), leading to ERK activation (Lange-Carter and Johnson, 1994; Wood et al., 1992). Rap1 was originally reported to antagonize Ras activation of c-Raf and ERK (Carey et al., 2003; Cook et al., 1993; Kitayama et al., 1989; Schmitt and Stork, 2001). Later, it was found that in tissues that express predominantly b-Raf (such as brain), Rap1 can activate ERK by activating b-Raf (Grewal et al., 2000; Ohtsuka et al., 1996; Wellbrock et al., 2004; York et al., 1998). Rap1 activation can also activate p38MAPK, which has been implicated in synaptic depression (Ahn et al., 2006; Bolshakov et al., 2000; Guan et al., 2003; Huang et al., 2004; Kanda and Watanabe, 2007; Sawada et al., 2001; Zhu et al., 2002). However, the intermediate steps are not clear. Similar to Rap1, Rap2 also counteracts Ras signaling by blocking c-Raf and subsequent ERK activation. In addition, Rap2 has been reported to activate c-Jun N-terminal Kinase (JNK), another member of the MAPK family, by interacting with its upstream element Tumor necrosis factor receptor-associated factor 2- and Nck-Interacting Kinase (TNIK) (Machida et al., 2004; Taira et al., 2004).

Another well-known effector of Ras family proteins is Phosphoinositide 3-kinase (PI3K), which is thought to regulate mTOR-dependent protein synthesis and actin rearrangement during memory processing (Horwood et al., 2006; Orme et al., 2006; Rodriguez-Viciana et al., 1994; Udo et al., 2005) (Figure 2B). Ras and Rap1, but not Rap2, have been reported to directly interact with the catalytic subunit of PI3K. This interaction requires Ras/Rap1 activation, and in turn, enhances the activity of PI3K and initiates its downstream signaling cascades (Rodriguez-Viciana et al., 1994, 1996a, 1996b; Sjölander et al., 1991). Interestingly, PI3K can regulate the activity of the Rho family of small G proteins, which plays significant roles in remodeling spine morphology in response to plasticity-related stimuli (Eickhoht et al., 2007; Graupera et al., 2008; Newey et al., 2005; Papakonstanti et al., 2007, 2008), suggesting a tight coordination between Ras family proteins with Rho family proteins in regulating synaptic plasticity.

Ras family proteins also interact with other families of proteins in the small G protein superfamily (Figure 2C). Activated Ras can turn on the GEF activity of Tiam1, leading to activation of Rac, a member of Rho family proteins (Lambert et al., 2002; Shirazi Fard et al., 2010; Yamauchi et al., 2005). In addition, Ras activation can lead to activation of RapGEF, which in turn activates Ral, a small G protein involved in exocytosis (Kikuchi and Williams, 1996; Urano et al., 1996; White et al., 1996), and Rin, which activates Rab5, a small G protein involved in endocytosis (Han and Colicelli, 1995; Tall et al., 2001). As will be discussed in the
following sections, the regulation of other small G proteins by Ras family proteins may also contribute to neuronal plasticity and memory formation.

**Regulation of Memory Formation and LTP/LTD by Ras Family Proteins**

In this section, we review a substantial body of evidence suggesting that signaling of Ras family proteins is critical for memory formation. These studies include investigation of (1) disruption of upstream regulators of Ras family proteins, (2) changes in the activity or amount of Ras family proteins, (3) disruption of their downstream effectors, and (4) knockout of related scaffolding proteins. In addition, long-lasting changes of synaptic efficacy, such as long-term potentiation (LTP) and long-term depression (LTD), have been closely associated with memory formation (Martin et al., 2000; Neves et al., 2008). Thus, many of the studies discussed here have also examined, in parallel, the engagement of Ras family proteins in these forms of synaptic plasticity.

**Upstream Regulators**

**Neurofibromin.** The first evidence suggesting a role of Ras in learning and memory comes from studies on NF1, an autosomal dominant disease caused by loss-of-function (LOF) mutations in neurofibromatosis type 1 oncogene ($nf1$). Thirty to sixty percent of children with NF1 have learning disabilities (North et al., 1997, 2002; Ozonoff, 1999; Riccardi, 1981). The $nf1$ gene encodes neurofibromin, a protein identified as a RasGAP (Figure 1B). LOF $nf1$ mutations result in decreased cycling from the active to inactive state of Ras, thus leading to Ras hyperactivity. This protein is universally expressed, but its expression level is highest in brain (Daston et al., 1992; Gutmann et al., 1991; Nordlund et al., 1995).

Mice with heterozygous $nf1$ knockout ($nf1^{+/−}$) were established as an animal model for NF1 disease by Jacks et al. (1994). Subsequently, Silva et al. (1997) found that these mice showed impaired spatial memory in the Morris Water Maze, a hippocampal-dependent memory task, suggesting a role of neurofibromin in the hippocampus during memory processing. Consistent with this idea, $nf1^{+/−}$ mice were able to overcome their performance deficit with extended training, which bypasses the requirement of the hippocampus (Packard and McGaugh, 1996; Pouzet et al., 2002). The impairment in spatial memory in $nf1^{+/−}$ mice was exacerbated with a further heterozygous mutation in the NR1 subunit of NMDA receptors (NMDARs), supporting the findings that the severity of learning disabilities in NF1 patients covaries with genetic backgrounds (Easton et al., 1993). A previous study has suggested that neurofibromin is expressed in many brain regions (Nordlund et al., 1995). However, Silva et al. (1997) did not observe any memory deficit of $nf1^{+/−}$ mice in cued fear conditioning, a form of amygdala-dependent and hippocampal-independent memory task, suggesting that neurofibromin does not affect amygdala processing of fear memory. It requires future study to know whether this lack of effect is due to differential expression levels of neurofibromin between the amygdala and the hippocampus. The role of neurofibromin in memory and oncogenesis can be dissociated. Mice carrying a homozygous knockout of exon 23a of $nf1$ ($nf1^{23a−/−}$), which encodes part of the GAP-related domain, showed no predisposition for tumor formation and developed normally. However, they exhibited impaired spatial memory (Costa et al., 2001). This study also highlights the indispensable role of the GAP activity of neurofibromin in memory formation.

Follow-up studies were carried out to explore the cellular and molecular mechanisms underlying memory impairment in $nf1^{+/−}$ mice. Costa et al. (2002) found that the spatial memory deficit in $nf1^{+/−}$ mice was rescued by a further heterozygous knockout in K-Ras or N-Ras. Pretraining injection of an FTase inhibitor or lovastatin, both of which inhibit membrane anchoring of Ras and thus disrupt Ras signaling, also rescued the memory impairment (Costa et al., 2002; Li et al., 2005). Consistent with behavioral findings, $nf1^{+/−}$ mice showed impaired LTP at hippocampal Schaffer collateral-CA1 synapses in response to theta-burst stimulation (TBS), which was rescued by inhibitors or LOF mutations of Ras. Furthermore, Guiding et al. (2007) found that basal ERK phosphorylation and downstream CREB phosphorylation was elevated in the hippocampus of $nf1^{+/−}$ mice, but there was no change in the PI3K cascade, suggesting that neurofibromin specifically suppresses the ability of Ras to activate the Raf-MEK-ERK-CREB cascade. Application of a subthreshold dose of a MEK inhibitor, which reversed the abnormal increase in ERK and CREB phosphorylation, reversed the LTP deficit in $nf1^{+/−}$ mice. Collectively, these findings suggest that the abnormal increase in Ras activity may account for memory impairment in $nf1^{+/−}$ mice.

Interestingly, Costa et al. (2002) found that in the presence of picrotoxin, a GABA antagonist, LTP was normally expressed in $nf1^{+/−}$ mice. Cui et al. (2008) went on to examine the role of neurofibromin in different cell types in the brain. Cell-specific heterozygous deletion of $nf1$ in GABAergic neurons, but not in excitatory neurons or astrocytes, resulted in deficits in spatial memory and hippocampal LTP, similar to those observed in $nf1^{+/−}$ mice. Consistent with these observations, GABA release was elevated in $nf1^{+/−}$ mice. Furthermore, a subthreshold dose of picrotoxin, which did not affect wild-type (WT) mice, rescued the memory deficit in $nf1^{+/−}$ mice. These data suggest that the engagement of neurofibromin in memory formation is cell type-specific: it restricts GABA release at inhibitory synapses and thus favors potentiation of synaptic strength.

Studies in *Drosophila* also suggest that neurofibromin plays important roles in learning and memory. In an aversive conditioning task, NF1-deficient flies showed both a learning deficit immediately after one-cycle training and a long-term memory deficit 24 hr after spaced repeated-trial training (Guo et al., 2000; Ho et al., 2007). Interestingly, these flies performed normally in memory tests after massed training, suggesting that the engagement of neurofibromin in memory formation is sensitive to the pattern of training trials. Neurofibromin has an N-terminal GAP-related domain, and a C-terminal region that regulates CAMP levels by mediating G protein-coupled receptor (GPCR) -dependent activation of adenylyl cyclase. Ho et al. (2007) found that the C-terminal region of neurofibromin was important for immediate memory, whereas its GAP activity was specifically required for the formation of long-term memory following spaced training.

In summary, these data suggest that hyperactive Ras induced by LOF mutations in neurofibromin, a specific RasGAP, can result in memory impairment. Despite the ubiquitous distribution
of neurofibromin, surprisingly, its engagement in memory formation is specific to (1) certain types of memory tasks, (2) GABAergic neurons (and not excitatory neurons or astrocytes), and (3) Ras-ERK cascade (and not Ras-PI3K cascade) (Figure 3A). Findings in Drosophila suggest that neurofibromin can also regulate learning by Ras-independent activation of adenylyl cyclase. This regulation has also been found in rodents (Tong et al., 2002), but its contribution to learning in rodents has not been reported. Furthermore, despite the importance of neurofibromin in cognitive function, little is known about how the activity of neurofibromin is regulated, especially by stimuli that give rise to memory formation and synaptic plasticity. Finally, it should be noted that in addition to learning disabilities, NF1 patients have many other cognitive deficits (North et al., 1997, 2002; Ozonoff, 1999; Riccardi, 1981). For example, most NF1 children are hyperactive and have attention problems, which are also observed in 

344 Neuron 68, November 4, 2010 ©2010 Elsevier Inc.

Figure 3. Regulation of Different Aspects of Synaptic Plasticity by GAPs and GEFs during Memory Formation

(A) Neurofibromin (NF1) regulates the release of inhibitory neurotransmitter, GABA, which binds to GABA receptors on dendritic shafts.

(B) SynGAP localizes in dendritic spines and regulates AMPAR-mediated LTP and LTD.

(C) RasGRFs also localize in dendritic spines and regulate AMPAR dynamics. In addition, there is also evidence that RasGRF1 can regulate excitability and the RasGRFs can regulate short-term presynaptic facilitation (see text); however, the underlying molecular mechanism is not clear.

(D) Epac can regulate both glutamate release in presynaptic terminals and AMPAR dynamics in postsynaptic spines.
in LTP (Kim et al., 2003; Komiyama et al., 2002). This deficit could not be rescued by a further knockout of H-Ras, suggesting that H-Ras may not be involved in SynGAP signaling. However, this study does not rule out the possibility of other Ras isoforms and other members in Ras family as SynGAP targets. Compared with a more general role of SynGAP in LTP, the engagement of SynGAP in LTD is specific to certain LTD-inducing stimuli. It was reported that in SynGAP+/− mice, LTD induced by 1 Hz stimulation is intact; however, LTD induced by NMDA treatment is significantly impaired (Carlisle et al., 2008; Kim et al., 2003). Finally, Rumbaugh et al. (2006) found that in cultured cortical neurons, SynGAP negatively regulated ERK signaling and positively regulated p38MAPK signaling. It also suppressed surface expression of AMPA receptors (AMPARs), postsynaptic glutamate receptors that are important for the expression of LTP and LTD. The effects of SynGAP were lost with LOF mutations in the GAP domain, suggesting that its GAP activity is crucial during the formation of different forms of synaptic plasticity, mostly likely acting through its regulation of the activity of Ras family proteins.

At the behavioral level, Komiyama et al. (2002) and Muhia et al. (2010) observed impairment in the formation of spatial memory of SynGAP+/− mice in the Morris Water Maze. However, both contextual and cued fear conditioning, as well as novel object recognition, appear to be normal in these mice (Muhia et al., 2009, 2010). In addition to spatial memory deficits, SynGAP+/− mice exhibited a range of behavioral and cognitive impairments, such as hyperactivity, reduction in anxiety, working memory deficit, and social isolation, as well as an impairment in controlling operant behaviors (Muhia et al., 2009, 2010). The complexity of the behavioral phenotype of SynGAP+/− mice makes it hard to interpret whether their impairment in memory tasks is due to deficits in learning and memory or to changes in performance variables such as arousal state.

Taken together, the current findings suggest an important role for SynGAP in hippocampal LTP and certain types of LTD, which may be mediated by Ras-ERK and Rap1-p38MAPK cascades (Figure 3B). Furthermore, SynGAP is critical for a variety of cognitive functions. However, to elucidate its specific function in learning and memory, conditional SynGAP knockouts in a spatially and temporally restricted fashion are required.

RasGRF. In contrast to neurofibromin and SynGAP, which suppress Ras activity, RasGRF1 and RasGRF2 are GEF proteins, promoting Ras activation. They are both abundantly expressed in brain (Chen et al., 1993; Shou et al., 1992). Activation of RasGRFs following increased intracellular Ca2+ concentration or GPCR activation leads to increased activity of both Ras and Rac, a small G protein in the Rho family (Fan et al., 1998; Innocenti et al., 1999) (Figure 1B). Thus, RasGRFs may coordinate the activation of Ras and Rac to change synaptic strength.

Brambilla et al. (1997) have generated a line of RasGRF1 knockout mice (RasGRF1−/−). Although RasGRF1 expression was clearly lost in both hippocampus and amygdala, these mice exhibited impaired memory only in amygdala-dependent tasks, such as inhibitory avoidance, and contextual and cued fear conditioning. However, spatial learning and memory, which critically rely on hippocampal function, but not amygdala, were normal. Consistent with their behavioral findings, amygdala LTP was impaired in these mice, whereas hippocampal LTP was unaffected. However, Giese et al. (2001) found opposite results with a different line of RasGRF1−/− mice: they showed impairment in hippocampal-dependent memory tasks, but not amygdala-dependent tasks. It is not clear what factors may contribute to the discrepancy in these findings. It may be due to the differences in the training protocols or the differences in the genetic backgrounds of these two lines of mice. Nevertheless, both studies suggest a role for RasGRF1 in memory formation. This general conclusion is further supported by a recent study by Fasano et al. (2009), who examined the same line of RasGRF1−/− mice used by Brambilla et al. (1997), as well as a line of mice mildly overexpressing RasGRF1 (RasGRF1OE). They found that hippocampus-dependent spatial memory in these mice was comparable to that in WT controls. However, passive avoidance learning requiring the amygdala was impaired in RasGRF1−/− mice and facilitated in RasGRF1OE mice. These findings are consistent with those from Brambilla et al. (1997). They also found that RasGRF1 was expressed in adult striatum and mediated ERK activation induced by dopamine or glutamate treatment. Given that striatum plasticity is significantly affected by drug addiction (Chang et al., 2007; Fasano and Brambilla, 2002), they explored the role of RasGRF1 in cocaine addiction. They found that the increase in locomotion following chronic cocaine treatment was impaired in RasGRF1−/− mice, and enhanced in RasGRF1OE mice. In addition, RasGRF1−/− mice exhibited impaired performance, whereas RasGRF1OE mice showed enhanced performance, in a conditioned place preference task, suggesting that RasGRF1 can also modulate maladaptive memory in drug addiction.

A number of studies have also examined memory formation and synaptic plasticity in RasGRF1 and RasGRF2 double knockout mice (RasGRF1−/−/RasGRF2−/−). For example, Li et al. (2006a) found that postpubescent RasGRF1−/−/RasGRF2−/− mice exhibited impaired LTP and LTD at hippocampal Schaffer collateral-CA1 synapses. Further examination using hippocampal slices from RasGRF single knockout mice revealed that RasGRF1 mainly regulates LTD by mediating the signaling between the NR2A subunit of NMDARs and p38MAPK activation. In contrast, RasGRF2 significantly contributes to LTP formation by linking NR2B activation to ERK activation. Interestingly, the deficits in LTP, but not LTD, in young RasGRF1−/−/RasGRF2−/− mice can be rescued by exposure of adolescent mice to an enriched environment (Li et al., 2006b). Early-age exposure to an enriched environment can also rescue contextual fear memory deficits in these mice (Arai et al., 2009). These findings suggest that the engagement of RasGRFs in memory formation is influenced by the previous experiences of animals during the adolescence.

RasGRFs also regulate electrophysiological properties of neurons other than LTP and LTD. Enhanced basal synaptic transmission in both amygdala and hippocampus has been reported in RasGRF1−/− mice (Brambilla et al., 1997). In addition, Tonini et al. (2001) reported that the excitability of hippocampal neurons was significantly increased in RasGRF1−/− mice. Finally, Li et al. (2006a) found that RasGRF1/2 double knockout or RasGRF2 knockout reduced paired-pulse facilitation (PPF) at
hippocampal Schaffer collateral–CA1 synapses, suggesting that RasGRF2 may also regulate presynaptically expressed short-term plasticity.

In summary, RasGRFs are important for regulating synaptic plasticity and intrinsic properties of neurons. Evidence suggests that RasGRFs regulate synaptic plasticity by transforming Ca2+ influx from NMDARs to activation of Ras family proteins and subsequent activation of ERK and p38MAPK signaling cascades (Figure 3C). The effects of RasGRFs on synaptic plasticity and neuronal function may likely underlie the observed role for these Ras-family regulators in memory.

Epac (cAMP-GEF). It is well known that cAMP can enhance neurotransmitter release (Chavez-Noriega and Stevens, 1994; Chen and Regehr, 1997; Zhong and Wu, 1991). Canonically, it is thought to exert its effect through protein kinase A (PKA) (Capogna et al., 1995; Trudeau et al., 1996). However, it was reported that cAMP-mediated synaptic facilitation was not completely eliminated by PKA inhibitors, suggesting the existence of other cAMP targets (Beaumont et al., 2002). Exchange Protein directly Activated by cAMP (Epac) was identified as a RapGEF that is activated by cAMP (de Rooij et al., 1998; Kawasaki et al., 1998) (Figure 1B). Thus, it was also called cAMP-GEF. cAMP binding to Epac leads to an increase in Rap1 and Rap2 activity, without affecting H-Ras or R-Ras, and the Rap activation was independent of PKA activity. Two isoforms of Epac have been identified. Epac1 is universally expressed, whereas Epac2 is predominantly expressed in the brain.

The investigation of the function of Epac has been greatly facilitated by the innovation of cAMP analogs that specifically activate either Epac or PKA. Presynaptic loading of an Epac-specific cAMP analog increases postsynaptic responses in the Calyx of Held in rats, suggesting a role for Epac in the regulation of neurotransmitter release (Kaneko and Takahashi, 2004). Similar results have been found in a number of other experimental systems (Cheung et al., 2006; Gekel and Neher, 2008; Zhong and Zucker, 2005). In mouse hippocampus, Epac activation enhances Schaffer collateral–CA1 LTP. A single train of 100 Hz stimuli usually only induces translation-independent early LTP, which decays within 30 min. However, in the presence of an Epac-specific cAMP analog, the induced LTP can last for at least 2 hr and is dependent on translation and ERK activity (Gelinas et al., 2008). In this study, a change in basal transmission or PPF by Epac-specific cAMP analog was not observed, implying that, in this instance, Epac may not be involved in presynaptic plasticity. Ster et al. (2009) reported that application of a higher concentration of Epac-specific cAMP analog to hippocampal slices caused synaptic depression that was dependent on Rap1 and p38MAPK signaling, but not on ERK. They also did not observe any change in PPF. Similarly, Woolfrey et al. (2009) found that Epac activation decreased AMPAR-mediated synaptic transmission in cultured rat cortical pyramidal neurons.

At the behavioral level, Ma et al. (2009) reported that bilateral infusion of cAMP analogs specific for either PKA or Epac to mouse dorsal hippocampus immediately after a weak training protocol for contextual fear conditioning enhanced long-term memory tested 24 hrs later. In addition, infusion of PKA inhibitors following a strong training protocol impaired memory, which could be rescued by Epac-specific cAMP analog. These data suggest a role for Epac in memory formation and suggest that Epac and PKA may have overlapping downstream targets during this process. Furthermore, cAMP is known to play important roles in hippocampus-dependent memory retrieval (Isiegas et al., 2008; Izquierdo et al., 2000). Dbh+/− mice, which are deficient in cAMP production, exhibit impairment in the retrieval of memory for contextual fear conditioning. Ouyang et al. (2008) found that infusion of general cAMP analogs, or a combination of PKA-specific and Epac-specific cAMP analogs to dorsal hippocampus before test, rescues the retrieval deficit in Dbh−/− mice. Interestingly, either PKA- or Epac-specific cAMP analog alone had no effect, suggesting a synergistic interaction between PKA and Epac signaling cascades during memory retrieval. The role for Epac in memory retrieval is further supported by a study performed by Ostroveanu et al. (2009): infusion of Epac-specific cAMP analog to dorsal hippocampus before test enhanced memory retrieval in contextual fear conditioning and passive avoidance. Moreover, infusion of Epac2 siRNA to knock down the major isoform of Epac in hippocampus blocked memory retrieval.

Collectively, Epac has been reported to be engaged in a number of forms of synaptic plasticity (Figure 3D), including the enhancement of neurotransmitter release, the facilitation of LTP, and the induction of synaptic depression. However, there are inconsistencies in the reports regarding where and how Epac contributes to plasticity, which may be due to differences in the composition of molecular networks in specific model systems or in the concentration and application time of Epac-specific cAMP analogs. In addition, Epac can recruit divergent signaling cascades (ERK and p38MAPK, respectively) in the service of synaptic potentiation versus synaptic depression. It will now be of interest to examine the factors that control this differential routing of Epac in the service of specific forms of synaptic plasticity. Furthermore, current studies on Epac in memory consolidation and retrieval have only used fear memory tasks, which are known to trigger neuromodulator release to generate cAMP in hippocampal neurons. This may implicate Epac as a critical element bridging the interactions between neuromodulatory systems and the hippocampus. It is not clear whether Epac has similar roles in nonemotional memory. It is also important to examine how the behavioral effects of Epac link to its synaptic and molecular functions. Finally, cAMP has also been reported to activate another GEF protein, CNRasGEF, which is also highly expressed in a variety of brain regions (Pham et al., 2000). Interestingly, CNRasGEF can activate both Ras and Rap1; however, its activation of Ras requires CAMP binding, while its activation of Rap1 is CAMP-independent. The role of CNRasGEF in memory formation and neuronal plasticity has not yet been examined.

Other Regulators. In addition to GAPs and GEFs, a few other proteins have recently been found to directly regulate the activity of Ras family proteins during memory formation.

Suprachiasmatic nucleus (SCN) circadian oscillatory protein (SCOP) was originally identified in SCN, and its expression is regulated by circadian rhythms (Shimizu et al., 1999). Later, it was found that SCOP is expressed in hippocampus. It localizes in lipid rafts, membrane microdomains that are highly packed with protein receptors and flanked by glycosphingolipids.
SCOP specifically binds to K-Ras, which is also present in lipid rafts. This interaction prevents K-Ras from binding to guanine nucleotides, thus holding K-Ras in the inactive state (Shimizu et al., 2003). Shimizu et al. (2007) reported that object recognition training in mice induced rapid degradation of SCOP in the hippocampus, in parallel with an increase in ERK activation. Pretraining overexpression of SCOP blocked the formation of long-term memory for object recognition without affecting short-term memory. These findings suggest that SCOP is an inhibitory constraint for long-term memory formation.

Sprouty-related protein with an EH1 domain (Spr1) is another inhibitor of Ras-Raf signaling, predominantly expressed in adult mouse brain (Engelhardt et al., 2004). It strengthens the binding between Ras and Raf, preventing Raf from being activated by Raf kinases (Wakioka et al., 2001). Denayer et al. (2008a) reported that Spr1 knockout mice (Spr1−/−) exhibited impaired spatial learning and memory in the Morris Water Maze and the T-maze. In parallel with behavioral deficits, these mice showed reduced basal synaptic transmission, which was especially pronounced at high stimulation strength. They also showed increased PPF at 10 and 20 ms interpulse intervals compared with those of WT controls, suggesting a change in presynaptic function. In addition, LTP was decreased whereas LTD was increased in Spr1−/− mice. At the molecular level, ERK activation in the hippocampus of Spr1−/− mice during LTP induction was significantly enhanced, suggesting that hyperactive Ras-ERK signaling might contribute to the changes in synaptic plasticity and memory formation in these mice.

Finally, Corkscrew (csw; Drosophila homolog of SHP-2) is a tyrosine phosphatase that transduces signals from receptor tyrosine kinases to activate Ras-ERK signaling, the precise, underlying mechanism of which is not clear (Lu et al., 1993; Matozaki et al., 2009; Oishi et al., 2006; Perkins et al., 1996). Pagani et al. (2009) have examined the role of csw in memory formation induced by different patterns of training in Drosophila. Interestingly, they found that the magnitude of csw activity determined the optimal spacing between training trials in an olfactory aversive conditioning task. In flies overexpressing WT csw, ERK was activated faster with a normal posttrial deactivation rate; this allowed memory to be induced by training with shorter intertrial intervals. In contrast, in flies expressing a gain-of-function (GOF) mutant csw, ERK was also activated faster, but the deactivation was delayed. In these flies, long-term memory could only be induced with longer intertrial intervals. This study suggests a role of csw-mediated Ras-ERK signaling in the spacing effect of memory formation.

Taken as whole, studies at the level of upstream regulators reveal an interesting point: either hyperactivation (by inhibiting negative regulators) or inhibition (by inhibiting positive regulators) of Ras family proteins can cause impairment in neuronal plasticity and memory formation. These data suggest that the signaling of Ras family proteins is required for neuronal and behavioral plasticity. However, elevation of these signaling cascades in inhibitory neurons (such as by neurofibromin knockout, Figure 3A) can result in memory impairment. Even in excitatory neurons, the signaling from Ras family proteins may require a particular dynamic range in order to integrate signals properly. Thus, if the signaling from Ras family proteins is elevated in the basal state, there may not be sufficient range available for it to be activated properly when responding to signals, thereby occluding memory formation. This appears to be a common theme in the nervous system, and it may be involved in a wide range of brain disorders (Hoeffer and Klann, 2010; Kelleher and Bear, 2008).

**Ras Family Proteins**

Ras. As described earlier, membrane association is critical for Ras signaling. A number of studies have examined the role of Ras in memory formation by local infusion of FTase inhibitors (which disrupt the attachment of Ras to membranes) into specific brain regions during different stages of memory processing. Merino and Maren (2006) found that pretraining infusion of an FTase inhibitor into basolateral amygdala (BLA), but not other amygdala subregions, disrupted long-term memory for contextual and cued fear conditioning. Intra-BLA infusion of the inhibitor postraining or prior to testing had no effect on long-term fear conditioning, suggesting a requirement of localized Ras signaling in BLA during the induction of fear memory. Similar findings have been reported by Ou and Gean (2006). At the cellular level, FTase inhibitors attenuate LTD in hippocampal dentate gyrus (Murray and O’Connor, 2004) and disrupt LTP in the hippocampal CA1 region (O’Kane et al., 2004). However, caution should be expressed in interpreting these results because (1) FTase inhibitors target a number of G proteins (thus, their phenotypic effects may not be due to specific inhibition of Ras signaling), and (2) FTase inhibitors target newly synthesized Ras without affecting preexisting functional Ras. Thus, a lack of effect of the inhibitors cannot completely exclude a contribution of Ras signaling to the process.

In addition, genetic manipulations have been utilized to examine the function of specific Ras isoforms in memory formation. Ohno et al. (2001) have combined pharmacological and genetic manipulations to study the role of K-Ras in plasticity. They found that K-Ras heterozygous knockout mice (K-Ras+/−) showed robust memory for contextual fear conditioning and normal hippocampal Schaffer collateral-CA1 LTP and ERK activation. However, a subthreshold dose of MEK inhibitor, which did not affect WT mice, blocked memory formation, LTP, and ERK activation in K-Ras+/− mice, revealing an engagement of K-Ras-ERK signaling in memory processing. Another Ras isoform, H-Ras, is abundantly expressed in CNS and relatively enriched in synapticosomes. Manabe et al. (2000) found that H-Ras knockouts (H-Ras−/−) mice showed increased tyrosine phosphorylation of NMDARs in hippocampus. In addition, NMDAR responses and hippocampal LTD induced by high-frequency stimulation (HFS) were larger, suggesting that H-Ras can restrain LTD induction by inhibiting NMDAR function. Interestingly, Komiyama et al. (2002) found that LTD induced by pairing low-frequency stimulation (LFS) with postsynaptic depolarization was normal in H-Ras−/− mice, suggesting that the engagement of H-Ras in LTD is sensitive to the stimulation patterns. Kushner et al. (2005) examined mice expressing a ca form of H-Ras (H-RasG12V) in forebrain postnatal neurons. Electron microscopy identified that H-RasG12V was predominantly localized in axon terminals of CA1 pyramidal neurons, and that H-RasG12V-containing axon terminals had more vesicles docking at active zones than did WT controls. H-RasG12V mice showed...
enhanced synaptic potentiation, even in the presence of NMDAR blockers. In addition, these mice exhibited enhanced acquisition of spatial memory in the Morris Water Maze, and enhanced short-term and long-term conditioned responses in contextual fear conditioning. The enhancement in synaptic potentiation and memory caused by H-Ras<sup>V12</sup> expression was reversed by knockout of Synapsin I, a protein involved in neurotransmitter release, suggesting that H-Ras can also contribute to memory formation by mediating presynaptic facilitation. Collectively, these studies suggest that the two Ras isoforms, K-Ras and H-Ras, regulate distinct aspects of synaptic plasticity during memory formation.

Rap1. Rap1 shares ~50% sequence identity with Ras. The ability of Rap1 to activate b-Raf, which is predominantly expressed in the brain and leads to persistent ERK activation, has been used as a target for the studies of molecular mechanisms underlying learning and memory (Storm et al., 1990; York et al., 1998). Morozov et al. (2003) generated a line of transgenic mice carrying inducible dominant-negative Rap1 (iRap) in the forebrain. Induced expression of iRap resulted in decreased b-Raf activity, but increased c-Raf activity, in hippocampus. The imbalance in Raf activity specifically reduced the activity of a membrane-associated pool of ERK, and decreased ERK-mediated phosphorylation of the A-type potassium channel, Kv4.2. In parallel with these molecular changes, iRap expression reduced cAMP-dependent forms of LTP and the channel, Kv4.2. In addition, these mice showed impaired extinction of contextual fear conditioning, which was accompanied by a reduction in ERK activation in hippocampus during early extinction trials. Collectively, these data suggest that Rap2 opposes ERK activity in Ras-ERK signaling in hippocampus, which may in turn regulate synaptic plasticity and cognitive function.

Overall, studies at the level of Ras family proteins provide direct and strong support for the hypothesis that the signaling of Ras family proteins is essential for regulating a variety of forms of neuronal plasticity in different brain regions, and that dynamic changes in their activity are crucial for the formation of many types of memories that are supported by plasticity within these same brain regions.

Major Downstream Effectors

b-Raf. Ras family proteins signal through Raf to activate the MEK-ERK signaling cascade (Figure 2A). There are three isoforms of Raf: a-Raf, b-Raf, and c-Raf. Among them, b-Raf has attracted special interest in neurobiology studies because it is predominantly expressed in the brain and is the major isoform of Raf in neurites (Storm et al., 1990). In addition, compared to other isoforms, b-Raf is a stronger MEK-ERK activator: it has higher affinity for MEK and mediates sustained ERK activation (Papin et al., 1996). To directly evaluate the role of b-Raf in memory processing, Chen et al. (2006) generated a line of conditional b-Raf knockout mice using the Cre-loxP system to restrictively knock out b-Raf expression in the postnatal forebrain neurons (b-Raf<sup>−/−</sup>). These mice exhibited impaired spatial learning and contextual discrimination, while contextual fear conditioning and conditioned taste aversion were spared. They also showed impaired hippocampal Schaffer collateral-CA1 LTP. At the molecular level, training-induced ERK activation in the hippocampus was significantly reduced. Collectively, these findings suggest a critical role of b-Raf in regulating hippocampal ERK activation, LTP, and memory formation.

PI3K. In contrast to Raf, PI3K activated by Ras family proteins phospholipids phosphatidylinositol lipid substrates, which in turn activate Akt (PKB) to regulate mTOR-dependent protein synthesis, a type of translation closely associated with cell growth. In addition, PI3K activation can lead to the activation of a number of GAPs and GEFs; thus, PI3K acts both upstream and downstream of Ras family proteins (Rodriguez-Viciana et al., 1996a; Vanhaesebroeck et al., 2010) (Figure 2B).

Lin et al. (2001) reported that pretraining infusion of PI3K inhibitors to lateral or basolateral amygdala (LA/BLA) impaired the induction of long-term, but not short-term, cued fear conditioning, and blocked training-induced Akt and CREB activation, suggesting that PI3K signaling is specifically required for long-term memory. In hippocampus, administration of PI3K inhibitors to the CA1 region impaired acquisition, consolidation, and retrieval of memory for inhibitory avoidance (Barros et al., 2001). It also impaired the retrieval and extinction, but not the acquisition or consolidation, of contextual fear conditioning (Chen et al., 2005). In addition, Mizuno et al. (2003) reported that chronic systemic injection of a PI3K inhibitor, wortmannin, impaired hippocampal-dependent spatial learning in a radial arm maze task. In contrast, Horwood et al. (2006) did not observe a requirement of PI3K in the same memory task using a different PI3K inhibitor, LY294002, whereas the inhibitor
impaired long-term memory for object recognition. It is not clear what factor or factors caused these contradictory results. One possibility is that different types, concentrations, and application time of the inhibitors used in these two studies might differentially affect Akt as opposed to other downstream effectors of PI3K, which in turn could result in different learning effects. Furthermore, Sui et al. (2008) recently found that inhibition of PI3K in medial prefrontal cortex (mPFC) immediately after training blocked long-term memory for trace fear conditioning. Finally, Tohda et al. (2007) examined knockout mice lacking the p85α regulatory subunit of PI3K, which showed reduced Akt phosphorylation in cerebral cortex, striatum, and hippocampal dentate gyrus and CA3 regions, but not in CA1 region. These mice exhibited impaired spatial learning as well as motivation deficits and restlessness in the Morris Water Maze task. Thus, PI3K signaling is important in various forms of memory as well as other cognitive functions.

At the cellular level, PI3K inhibitors block amygdala LA-BLA LTP, as well as Akt and ERK activation induced by tetanic stimulation, suggesting a linkage of PI3K to ERK signaling in synaptic plasticity (Lin et al., 2001). HFS-induced LTP in mPFC and tetanus-induced LTP in hippocampal dentate gyrus were also impaired in the presence of PI3K inhibitors (Horwood et al., 2006; Kelly and Lynch, 2000; Sui et al., 2008). At hippocampal Schaffer collateral–CA1 synapses, Sanna et al. (2002) reported that the expression but not the induction of LTP induced by HFS was blocked by a PI3K inhibitor. However, Opazo et al. (2003) reported opposing findings in which PI3K inhibitors partially blocked LTP induction without affecting its expression. The contradiction might be due to differences in species, drug concentration, or stimulation protocol. Opazo et al. (2003) also found that LTP induced by theta-pulse stimulation (TPS) was suppressed by PI3K inhibitors and ERK inhibitors, whereas LTP induced by pairing presynaptic stimulation with postsynaptic depolarization only required PI3K activity, suggesting that PI3K and ERK signaling differentially contribute to LTP induced by different patterns of stimulation. Furthermore, PI3K activity is required for the persistence of late-phase LTP induced by multiple trains of HFS or TBS (Karpova et al., 2006; Raymond et al., 2002). Finally, PI3K-Akt-mTOR signaling was found to be engaged in metabotropic glutamate receptor (mGluR)-dependent LTD (Banko et al., 2006; Hou and Klann, 2004).

In addition to its role in mammalian systems, PI3K also plays important roles in synaptic plasticity in invertebrates. Its activity is required for long-term synaptic facilitation in crayfish and Aplysia (Beaumont et al., 2001; Hu et al., 2006). Interestingly, a recent study in Drosophila found that Aβ42, which forms plaques in Alzheimer disease, induced PI3K hyperactivity, whereas Aβ42-induced memory loss was rescued by PI3K inhibition (Chiang et al., 2010). This study suggests that, similar to ERK, hyperactive PI3K can also lead to memory impairment.

Rin1. Rin1 is a GEF for Rab5, a small G protein of the Rab family involved in endocytosis of surface receptors, including AMPARs (Brown et al., 2005; Tall et al., 2001). Active Ras can bind to and enhance the activity of Rin1 (Han and Colicelli, 1995) (Figure 2C). Rin1 also competes with c-Raf for Ras binding (Han and Colicelli, 1995). Thus, it is thought to antagonize the effects of the Ras-Raf-MEK-ERK cascade in memory processing. Rin1 is highly expressed in the brain, relatively enriched in hippocampus, amygdala, striatum, and forebrain cortex, and is localized in the soma and dendrites of neurons (Dhaka et al., 2003). Dhaka et al. (2003) found that Rin1 knockout mice (Rin1<sup>−/−</sup>) exhibited enhanced short-term and long-term memory for cued fear conditioning and conditioned taste aversion. Because the amygdala is essential for both tasks, they also examined amygdala LTP in these mice and found that the magnitude of LTP was increased. Knockout of Rin1 did not affect hippocampal LTP or hippocampus-dependent spatial learning and memory, suggesting a brain region (amygdala)-specific role of Rin1 in memory processing. A recent study examined fear extinction and latent inhibition in Rin1<sup>−/−</sup> mice (Bliss et al., 2010). In this study, control and knockout mice were extensively trained to show comparable strong levels of memory for cued fear conditioning, and afterwards, extinction trials were delivered. Twenty-four hours after extinction training, WT controls showed a significant reduction in conditioned fear responses. However, conditioned responses in Rin1<sup>−/−</sup> mice remained the same as their pre-extinction levels. Furthermore, latent inhibition, in which in WT animals pre-exposure of a cue impairs subsequent associative learning, was impaired in Rin1<sup>−/−</sup> mice. Depotentiation has been proposed as mechanism for fear extinction (Kim et al., 2007). However, Rin1<sup>−/−</sup> mice exhibited enhanced depotentiation in amygdala LA-BLA synapses as compared with WT mice, arguing against depotentiation as the mechanism mediating extinction. It remains to be examined whether plasticity in other amygdala circuits might account for the behavioral impairments.

In summary, the studies examining the effectors of Ras family proteins further support the model that signaling of Ras family proteins plays essential roles in multiple forms of memory and synaptic plasticity in a variety of brain regions. However, the engagement of each downstream effector of these G proteins in the induction of plasticity appears to depend on the brain regions involved, as well as the type of memory task and pattern of stimulation utilized. It should also be noted that although Ras family proteins play significant roles in activating these effectors, the effectors can also be activated independent of Ras family proteins (Rodríguez-Viciána et al., 1996a; Vanhaesebroeck et al., 2010; Wellbrock et al., 2004). Thus, further evaluation is required to determine whether the specific changes in both memory and synaptic plasticity caused by blocking these downstream effectors are uniquely due to disruption of the signaling from Ras family proteins.

**Scaffolding Proteins**

As implied in the discussion above, Ras family proteins interact with many upstream and downstream elements. While Ras family proteins appear to be engaged in multiple types of neuronal plasticity in a variety of brain regions, changes in a specific upstream or downstream element can produce highly localized effects. How does this specificity occur? One potential answer can be derived from the function of different scaffolding proteins on the signaling network, which can bring a subset of molecular elements into physical proximity, allowing specific interactions to occur. Two recent studies have directly assessed the role of these scaffolding proteins in memory formation.
Kinase Suppressor of Ras1 (KSR) is a scaffolding protein for the Ras-ERK cascade. It is highly expressed in adult brain, especially in the hippocampus. Behavioral analysis of KSR1 knockout mice (KSR1−/−) showed that these mice had deficits in a variety of memory tasks, including contextual fear conditioning, cued fear conditioning, passive avoidance, and spatial memory (Shalin et al., 2006). Furthermore, TBS-induced LTD at hippocampal Schaffer collateral-CA1 synapses was lost in KSR1−/− mice. There was also a decrease in the spiking of CA1 neurons during the first train of TBS. Interestingly, in KSR1−/− hippocampus, ERK activation induced by activators of PKC or mGluR, but not forskolin (an activator of cAMP signaling), was impaired. Specificity also remains downstream. ERK-dependent phosphorylation of Kv4.2, but not ribosomal S6 kinase (RSK), induced by the PKC activator was blocked. These findings suggest (1) that KSR1 specifically links mGluR/PKC activation to the Ras-ERK cascade and to downstream regulation of Kv4.2; and (3) that the mGluR/PKC-Ras-ERK-Kv4.2 signaling cascade is essential for memory formation.

Another scaffolding protein, Downstream of Receptor Kinase (DRK; Drosophila homolog of GRB2), mediates the interaction between Ras and receptor tyrosine kinases. Moressis et al. (2009) examined the role of DRK in memory formation using Drosophila as a model system. In Drosophila, DRK is preferentially expressed in axons of the projection neurons of the mushroom bodies, an integrative brain region critical for learning and memory. DRK heterozygous LOF mutant flies exhibited impaired acquisition and consolidation of olfactory aversive conditioning. Moreover, acute expression of ca-Ras or WT-Raf in the mushroom bodies allowed the mutant flies to acquire the task and exhibit immediate memory; however, the memory decayed within 90 min. These data suggest that scaffolding signaling of receptor tyrosine kinase-Ras-Raf is important for the acquisition of olfactory aversive conditioning. Specifically, the removal of AMPARs from synapses weakens synaptic strength. We then examine a role of Ras family proteins in regulating structural changes, which are thought to be involved in retention of long-term memory. Finally, we review a number of studies examining changes in Ras family proteins in response to training that induce memory formation and synaptic plasticity.

**Cellular and Molecular Mechanisms Recruited by Ras Family Proteins during Memory Processing**

As discussed above, although there are occasional inconsistencies in specific aspects of plasticity regulated by Ras family proteins, the aggregate evidence from a wide range of studies in several model systems strongly suggests that dynamic changes in these proteins are critical for memory formation and neuronal plasticity. Thus, in this section we review studies examining the cellular and molecular machinery recruited by Ras family proteins in the service of neuronal plasticity, which may in turn regulate memory formation at the behavioral level. We first discuss how Ras family proteins function both in postsynaptic sites and presynaptic sites to regulate synaptic strength. We then examine a role of Ras family proteins in mediating structural changes, which are thought to be involved in retention of long-term memory. Finally, we review a number of studies examining changes in Ras family proteins in response to training that induce memory formation and synaptic plasticity.

**Regulation of Postsynaptic Receptors**

AMPARs. Changes in AMPAR trafficking are important components of synaptic plasticity, and have been implicated in multiple forms of adaptive behavior, including learning and memory (Kessels and Malinow, 2009). It is commonly thought that insertion of AMPARs into the postsynaptic membrane enhances synaptic strength, which underlies the expression of LTP. Conversely, the removal of AMPARs from synapses weakens synaptic strength, leading to LTD or depotentiation.

To understand the role of Ras in AMPAR dynamics, Zhu et al. (2002) transfected hippocampal CA1 pyramidal neurons with WT-, ca- or dominant negative (dn)-Ras (H-Ras sequence was used in this study) and examined AMPAR current by whole-cell patch recording at −60 mV. Overexpression of WT- or ca-Ras enhanced whole-cell response mediated by AMPAR at the basal state, whereas dn-Ras reduced the responses. Furthermore, they found that dn-Ras suppressed AMPAR responses by inhibiting synaptic insertion of AMPARs containing GluR2L, an AMPAR subunit with a long cytoplasmic tail, and ca-Ras enhanced AMPAR responses by promoting insertion of GluR1-containing AMPARs. To probe the role of Ras in long-term plasticity, Zhu et al. (2002) induced LTD by pairing presynaptic stimulation with postsynaptic depolarization, and induced LTD by LFS. They found that Ras activation and subsequent ERK activation was required for LTD in AMPAR current, but not for LTD, supporting the model that Ras promotes synaptic insertion of AMPARs during LTD. To further elucidate the intermediate steps between Ras and AMPARs, Qin et al. (2005) transfected hippocampal slices with different ca-Ras mutants, targeting specifically c-Raf, b-Raf/RalGEF, or PI3K. They found that c-Raf was not required in AMPAR insertion, whereas b-Raf-MEK-ERK signaling was both necessary and sufficient for synaptic insertion of GluR2L-containing AMPARs during spontaneous activity. However, insertion of GluR1-containing AMPARs required a synergistic interaction between neuromodulatory signaling and neuronal activity to activate both b-Raf-MEK-ERK and PI3K-Akt cascades. In addition, Hu et al. (2008) reported that the impairment in GluR1-dependent LTD associated with Fragile X syndrome could be rescued by overexpression of WT-Ras or by reducing the threshold of PI3K-Akt activation by blocking an inhibitor in the cascade.

In contrast to Ras, Zhu et al. (2002) found that overexpression of WT-Rap1 or ca-Rap1 resulted in a decrease in AMPAR response, whereas dn-Rap1 expression enhanced AMPAR responses. The decrease in AMPAR responses by ca-Rap1 was mediated by p38MAPK-dependent removal of GluR2-containing AMPARs from postsynaptic membranes. Furthermore, dn-Rap1 blocked LTD in AMPAR current, which involved removal of GluR2-containing AMPARs, whereas LTD was not affected. In support of the idea that Rap1 activation promotes AMPAR removal, Cingolani et al. (2008) reported that Arginine-Glycine-Aspartic Acid peptides (RGD-peptides, which disrupt integrin signaling) induced Rap1 activation, and in parallel, a reduction of mEPSC amplitude through internalization of GluR2-containing AMPARs. dn-Rap1 expression reversed the RGD-induced reduction in mEPSC amplitude.

Similar to Rap1, Zhu et al. (2005) found that Rap2 activation also led to removal of AMPARs from synapses. However, unlike Rap1, Rap2 activation activated JNK, but not p38MAPK or ERK. Inhibition of JNK or an upstream activator of JNK blocked Rap2-mediated depression of AMPAR response. Inhibition of Rap2 or
JNK activation also blocked AMPAR-mediated depotentiation. Furthermore, ca-Rap2 expression caused removal of GluR1- and GluR2L-containing AMPARs from synapses. Finally, blocking Rap2 activation resulted in enhanced basal GluR2L phosphorylation, and increased GluR1 phosphorylation induced by ca-Ras expression, suggesting that Rap2 removal of AMPARs involves activation of a protein phosphatase.

Collectively, these findings suggest that Ras, Rap1, and Rap2 control different steps of AMPAR trafficking by recruiting distinct signaling cascades. During spontaneous activity, the recycling of GluR2L-containing AMPA receptors is bidirectionally controlled by Ras-ERK and Rap2-JNK cascades, and the insertion of GluR2-containing AMPARs is suppressed by the Rap1-p38MAPK cascade. In response to LTP-inducing stimuli, Ras activates both ERK and PI3K cascades to drive GluR1-containing AMPARs into synapses. In contrast, during LTD, Rap1 removes GluR2-containing AMPARs by activating p38MAPK. At already-potentiated synapses, LFS can activate the Rap2-JNK cascade to remove GluR1-containing AMPARs, resulting in depotentiation.

However, these findings contradict a number of reports that we have discussed in the previous section: (1) Manabe et al. (2000) found that H-Ras knockout mice exhibited larger LTP by removing the inhibition on NMDARs, but not affecting AMPARs. Here, Zhu et al. (2005) found that dn-H-Ras expression blocked AMPAR insertion and LTD. A caveat of the study by Zhu et al. (2005) is that dn-H-Ras was expressed with C-terminal attachment of green fluorescent protein (GFP), which may affect the specific subcellular localization of dn-H-Ras (Omerovic and Prior, 2009; Zhang and Casey, 1996). Thus, the blockade of AMPAR insertion may not be caused by inhibiting H-Ras, but rather by disrupting the signaling from other Ras isoforms, such as K-Ras, which has been found to be critical for LTP (Ohno et al., 2001). (2) Morozov et al. (2003) found that dn-Rap1 expression reduced LTD induced by TBS, whereas Zhu et al. (2005) reported that dn-Rap1 expression did not affect LTD induced by a pairing protocol. A reason for the contradictory results may reside in the different LTD induction protocols that are used in these studies. In fact, in the study by Morozov et al. (2003), Rap1 was recruited in LTD induction when cAMP signaling was triggered. They found that LTD induced by multiple HFS, which is cAMP independent, was not blocked by dn-Rap1. (3) Ryu et al. (2008) found that ca-Rap2 expression enhanced LTD induction and decreased ERK activity without affecting depotentiation and JNK, which are opposite to the findings by Zhu et al. (2005). One possible explanation is that in the study by Ryu et al. (2008), Rap2 activity was chronically elevated, which may induce compensatory mechanisms that change the rules for the induction of synaptic plasticity.

NMDARs. NMDARs are dually regulated channels that allow influx of Ca$^{2+}$ in response to a combination of glutamate binding and membrane depolarization. Ca$^{2+}$ influx from NMDARs plays an essential role in the induction of a variety of forms of LTP and LTD. Although changes in the activity of Ras family proteins do not affect NMDARs during spontaneous activity (Zhu et al., 2002), several studies suggest that H-Ras signaling can regulate the function of NMDARs during the induction of long-lasting synaptic plasticity.

Thornton et al. (2003) treated brain slices with Tat-H-Ras (Tat is a cell-permeable peptide) and found a decrease in the tyrosine phosphorylation and membrane retention of the NR2A subunit of NMDARs. They also detected a decrease in the activity of a cytosolic tyrosine kinase, Src. The effect of Tat-H-Ras on NMDARs was blocked in slices from Src knockout mice. Furthermore, consistent with the notion that NR2A-containing NMDARs are critical for LTP induction, Tat-H-Ras significantly reduced hippocampal Schaffer collateral CA1 LTP. In another study, Suvarna et al. (2005) found that internalization of NR2A-containing NMDARs induced by acute ethanol exposure was blocked in the presence of Tat-dn-H-Ras. These findings are consistent with the earlier report by Manabe et al. (2000), in which H-Ras knockout resulted in larger LTD, collectively suggesting that H-Ras activation can reduce surface retention of NR2A-containing NMDARs by inhibition of Src.

**Regulation of Presynaptic Neurotransmitter Release**

In addition to modifications of postsynaptic receptors, changes in synaptic strength can also be achieved by regulating neurotransmitter release in presynaptic terminals. Presynaptic plasticity has been strongly implicated in learning and memory in invertebrate systems, such as Aplysia (Kandel, 2001). In mammals, LTP at hippocampal Schaffer collateral-CA1 synapses, which is the most commonly studied form of LTP in the field of learning and memory, is thought to be largely due to postsynaptic modifications. However, presynaptically expressed LTP has been identified in a variety of brain regions, including cerebellar parallel fiber-Purkinje cell synapses, corticothalamic synapses, and hippocampal mossy fiber-CA3 synapses, and may contribute to information processing and storage in these regions (Powell, 2006).

A role for Ras family proteins in presynaptic plasticity has been referred to several times in previous sections of this review. For example, neurofibromin, a RasGAP, restricts GABA release from inhibitory neurons and is important for memory formation (Costa et al., 2002; Cui et al., 2008) (Figure 3A). On the other hand, ca-H-Ras expression enhances synaptic potentiation and memory formation by facilitating vesicle docking and release in excitatory neurons (although it remains to be established whether H-Ras is engaged in presynaptic plasticity under natural signaling conditions) (Kushner et al., 2005). Finally, at corticofugal synapses, Rap1 suppresses neurotransmitter release during the formation of fear memories (Pan et al., 2008). In addition, hints of Ras family proteins regulating presynaptic plasticity come from studies focused on two specific downstream signaling elements: Rap and Synapsin I.

**Raf.** Raf is a small G protein present at high levels at synaptic vesicle membranes. Active Ras and Rap1 can both activate Raf-GEFs by recruiting them to their target Raf in the membrane or by enhancing the catalytic activity of Raf-GEFs via PI3K (Feig, 2003) (Figure 2C). A number of studies suggest that Raf plays an important role in regulating neurotransmitter release. Polzin et al. (2002) reported that Sec6/8, which targets secretory vesicles to release sites in plasma membranes, bound specifically to active Raf. Furthermore, in synaptosomes prepared from mouse cortical neurons, dn-RafA expression blocked the enhanced glutamate release induced by a PKC activator without affecting basal release. It also reduced the refilling of the readily releasable
pool of synaptic vesicles after Ca\(^{2+}\)-triggered depletion. In another study, Owe-Larsson et al. (2005) blocked Rap signaling in cultured hippocampal neurons by either pharmacological inhibitors or expression of dn-RaIA, and observed a significant reduction in neurotransmitter release probability. Thus, Ras/Rap1 may regulate neurotransmitter release by activating Rap. However, it has not been elucidated whether Rap activity is required for memory formation. In addition, because Ras-independent mechanisms of RapGEF activation exist, it is not clear whether Rap-mediated regulation of neurotransmitter release is uniquely under the control of Ras/Rap1.

**Synapsin I.** Synapsin I is a protein that is associated with the surface of synaptic vesicles and is critical for maintaining a reserve pool of synaptic vesicles and regulating the rate of vesicle release (Hilfiker et al., 1999). The C terminus of Synapsin I contains consensus sites for phosphorylation by ERK, which is a major downstream effector of Ras family proteins. In synaptosomes prepared from rat or mouse cortex, the enhancement of neurotransmitter release induced by BDNF requires ERK phosphorylation of Synapsin I (Jovanovic et al., 2000). Another independent study found that AMPA stimulated ERK activation and subsequent Synapsin I phosphorylation in synaptosomes that occurred in parallel with a significant increase in synaptic vesicle recycling rate (Schenk et al., 2005). Recently, Vara et al. (2009) examined the role of the ERK-Synapsin I cascade in synaptic plasticity at hippocampal mossy fiber-CA3 synapses. They found that posttetanic potentiation was enhanced by ERK inhibition. The enhancement was lost in mice lacking Synapsin I, suggesting an inhibitory role of ERK-Synapsin I signaling in this form of plasticity. Furthermore, in Aplysia, ERK has been reported to mediate the effect of transforming growth factor \(\beta1\) (TGF-\(\beta1\)) on reducing synaptic depression through phosphorylation and redistribution of Synapsin I (Chin et al., 2002). Collectively, these studies suggest that Ras family proteins may contribute to presynaptic plasticity through regulation of the ERK-Synapsin I cascade.

**Regulation of Structural Plasticity**

The formation of long-term synaptic plasticity and long-term memory is closely associated with the remodeling of synaptic structure. Learning experiences that give rise to long-term memory have been found to induce increases in the number of presynaptic varicosities as well as increases in postsynaptic spine area and density, whereas mental retardation is often associated with synapse retraction and elimination. While its exact function is still under debate, this structural plasticity has been proposed to serve as a physical basis for modulating memory capacity or storage (Bailey, 1999; Bourne and Harris, 2008; Segal, 2005). Ras family proteins have long been found to regulate neuronal morphology during development by mediating the signaling of growth factors. Recently, studies suggest that they also contribute to structural plasticity associated with the formation of long-term synaptic and behavioral plasticity.

The first line of evidence comes from the analyses of Syn-Ras mice, transgenic mice expressing ca-H-Ras in postmitotic neurons in which synaptic contacts have been mostly established (Heumann et al., 2000). Overall, these mice showed an increase in neuronal structural complexity that was correlated with an increase in efficacy of synaptic transmission (Alpár et al., 2003, 2004; Arendt et al., 2004; Gärtner et al., 2004a, 2004b; Heumann et al., 2000; Seeger et al., 2004, 2005). However, in these studies, ca-H-Ras was expressed for months prior to the analysis of the structural changes, while learning events typically happen in a much more restricted temporal window. Thus, it is not yet clear whether these findings can be unequivocally implicated as mechanisms contributing to synaptic plasticity rather than simply reflecting postnatal developmental processes.

Ras family proteins have also been reported to interact with molecular elements engaged in structural plasticity. Dynamics of actin filaments in spines have been implicated as important elements in spine remodeling (Carlisle and Kennedy, 2005). Drebrin is a protein critical for disassembly of actin filaments (Hayashish and Shirao, 1999; Takahashi et al., 2003). Biou et al. (2008) found that the increase in spine motility induced by Drebrin overexpression could be reversed by blocking Rap activation. Interestingly, ca-Ras expression induced a similar increase in spine motility, which was reversed by knockdown of Drebrin. These data suggest that there are reciprocal interactions between Ras and Drebrin during spine turnover. Rap1 can also regulate structural dynamics of spines through interaction with actin filaments. In cultured cortical neurons, activation of Rap1 through NMDAR activation, or activation of Epac2, a RapGEF, decreases average spine area and increases spine motility (Wooolfrey et al., 2009; Xie et al., 2005). In contrast, overexpression of SPAR, a PSD-enriched RapGAP, results in actin filament reorganization and spine enlargement (Figure 1B). These effects of SPAR on spine morphology require its RapGAP activity and actin-interacting domains (Pak et al., 2001). With respect to downstream signaling elements linking Rap1 activation to actin rearrangement, activated Rap1 has been found to target AF-6, a synaptic actin-binding protein, to PSD puncta. Moreover, the PDZ-binding domain of AF-6 is required for Rap1-induced spine turnover (Srivastava et al., 2008; Xie et al., 2005).

Growth of new synapses also requires dissociation and reassociation of cell adhesion molecules, a group of membrane-associated proteins that interact with the extracellular matrix. Neuronal cell adhesion molecule (NCAM) signaling has been implicated in memory consolidation and long-term synaptic facilitation (Bailey et al., 1992; Hoffman, 1998). Moreover, NCAM clustering has been reported to induce the assembly of the PSD (Sytnyk et al., 2006). Schmid et al. (1999) found that NCAM clustering activated the Ras-ERK-CREB signaling cascade. In turn, the Ras-ERK signaling cascade activated tissue plasminogen activator (tPA), which degrades NCAM. These reciprocal interactions between NCAM and Ras promoted neurite outgrowth (Son et al., 2002). Furthermore, Koh et al. (2002) have identified an interaction between Ras-ERK signaling cascades and another cell adhesion molecule, Fasciclin II. They found that in the *Drosophila* larval neuromuscular junction, overexpression of Ras increased the number of presynaptic boutons by reducing the level of synaptic Fasciclin II.

Collectively, these findings suggest that Ras family proteins can regulate synapse structure through two mechanisms: (1) remodeling of actin filaments, and (2) degradation of cell adhesion molecules. These findings open the door for future research into the precise mechanisms by which Ras family proteins modulate structural plasticity.
Dynamics in Ras Family Proteins Induced by Plasticity-Related Stimuli

To fully understand how Ras family proteins are engaged in memory formation and neuronal plasticity, it is important to examine how stimuli that give rise to neuronal and behavioral plasticity can induce correlated changes in Ras family proteins.

Toward that end, a number of studies have examined the activation of Ras family proteins by Ca\(^{2+}\), a critical element in activity-dependent plasticity. Rosen et al. (1994) first demonstrated that membrane depolarization of cultured cortical neurons by potassium chloride induced rapid and transient Ras activation, and subsequent activation of MEK and ERK. The activation required Ca\(^{2+}\) influx from voltage-gated Ca\(^{2+}\) channels. Farnsworth et al. (1995) further showed that depolarization-induced Ras activation required calmodulin binding to a GEF protein, RasGRF. Ras has also been found to be activated upon NMDAR activation (Yun et al., 1998). In addition, membrane depolarization can activate Rap1. Grewal et al. (2000) found that Ca\(^{2+}\)-induced Rap1 activation required PKA activity and led to association of Rap1 with b-Raf. These studies suggest, albeit indirectly, that neuronal activity during memory processing activates Ras family proteins.

Neuronal activation can also regulate the distribution of Ras family proteins. Fivaz and Meyer (2005) found that in cultured hippocampal neurons, glutamate treatment induced reversible translocation of K-Ras and Rap1, but not H-Ras, from plasma membrane to perinuclear membrane compartments. This translocation required Ca\(^{2+}\) influx, activation of NMDARs, and an interaction with calmodulin. They also showed that the translocation of K-Ras did not inhibit K-Ras activity, but rather, it redistributed K-Ras activity to intracellular membrane compartments. This translocation of K-Ras and Rap1 upon neuronal activation may be an important vehicle for synapses to communicate with the nucleus.

Finally, a few very recent studies have examined the correlation between dynamics in the activity of Ras family proteins and the formation of synaptic and behavioral plasticity. Eckel-Mahan et al. (2008) found a circadian oscillation in Ras activity and downstream ERK activity in mouse hippocampus, which peaked during the day and declined at night. In parallel with the molecular oscillation, mice trained in a contextual fear conditioning task during the day showed robust long-term memory, whereas mice trained or tested at night exhibited only short-term memory. In addition, infusion of ERK activation inhibitors into hippocampus during the circadian peak, but not during the trough of ERK activation, impaired long-term memory for contextual fear conditioning. These data revealed a temporal correlation between Ras-ERK activity in hippocampus and long-term memory formation (Figure 4A).

In another study, Harvey et al. (2008) examined the dynamics of Ras activity in single spines of pyramidal neurons in hippocampal slices. They used a two-photon laser to locally uncage glutamate at the target spine while imaging Ras activity with a FRET-based indicator. They found that a train of excitatory laser pulses that uncage glutamate induced sustained enlargement of the stimulated spine, but not of neighboring spines. The train also induced robust Ras activation in the stimulated spine, the magnitude of which was correlated with spine enlargement. The activation of Ras was transient, peaking within 1 min and returning to baseline by 15 min. Interestingly, the activated Ras spread over several micrometers and invaded 10–20 neighboring spines. This spread of Ras activity allowed subthreshold stimuli applied in temporal registry at neighboring spines to also induce sustained spine enlargement, suggesting an important role of the spatial distribution of Ras activity in mediating synaptic sharing of LTP (Figure 4B).

Ye et al. (2008) recently analyzed Ras, Rap1, and ERK activation simultaneously in response to different patterns of stimulation [either spaced repeated-trial serotonin (5-HT), massed 5-HT pulses, or a single 5-HT pulse combined with neuronal depolarization], which induces mechanistically distinct forms of synaptic facilitation in Aplysia (Mauelshagen et al., 1998; Sutton et al., 2001, 2002, 2004; Sutton and Carew, 2000). Ye et al. (2008) found that Ras and Rap1 could serve either as activators or as inhibitors of ERK, depending upon the specific pattern of stimulation. Furthermore, Ras and Rap1 were functionally interactive during the formation of synaptic plasticity. In response to stimuli that induce long-lasting synaptic facilitation, the magnitude of ERK activation was regulated by the specific balance between Ras and Rap1 activation, rather than by the individual activation of either one alone. This functional interaction was absent in response to massed 5-HT, a pattern that does not induce lasting synaptic plasticity or long-term memory (Mauelshagen et al., 1998; Sutton et al., 2002). Recent findings also suggest that pattern-sensitive regulation of ERK by interactive Ras and Rap1 may contribute to the molecular routing of ERK to specific cellular compartments and give rise to downstream signaling (PKA versus PKC) that mediates the expression of specific, pattern-sensitive forms of synaptic plasticity and memory (Shobe et al., 2009) (Figure 4C).

Conclusions and Perspectives

Memory, the ability to encode, store, and retrieve information about the world, is a cornerstone of adaptive behavior, important for the survival of individual animals and for the evolution of the animal kingdom. A critical step in the molecular processing underlying memory formation is the conversion of extracellular stimuli arriving at neuronal surfaces into intracellular signaling cascades that modify synaptic connections and neuronal excitability, ultimately leading to the storage of information. The data we present in this review suggest the view that Ras family proteins critically regulate synaptic plasticity and memory formation.

An overview of the rich body of evidence in this review reveals a number of critical features of the signaling of Ras family proteins that have given them this important position in memory processing. First, Ras family proteins are ubiquitously expressed in brain, regulating information processing in many brain regions. They are also interconnected with a large molecular network of upstream and downstream signaling elements, thus having the capacity for integrating a great variety of extracellular stimuli during different forms of learning experiences and regulating
different aspects of neuronal plasticity to achieve memory formation.

Second, despite its extreme complexity, the signaling network of Ras family proteins is very well organized, through (1) interaction of multidomain GEFs and GAPs with specific signaling messengers and structural proteins, and (2) clustering of multiple signaling elements by scaffolding proteins. These provide a mechanism for specificity in information processing. An example is shown in Figure 3: different GEFs and GAPs are able to differentially regulate specific forms of synaptic plasticity in distinct cellular compartments, and recruit divergent downstream signaling cascades. In addition, the engagement of specific regulators or effectors of Ras family proteins in memory formation is highly sensitive to the type of memory task and specific pattern of training. Furthermore, activation of different members in Ras family can lead to distinct downstream signaling cascades. From this point of view, differential combinations of Ras family proteins and related signaling elements can encode different learning experiences.

Third, the activity of Ras family proteins during memory processing is highly dynamic. Studies show that hyperactivation or inhibition of Ras family proteins result in deficits in memory formation and neuronal plasticity, suggesting that memory strength is not determined by the absolute magnitude of the activation of Ras family proteins. Recent studies of the changes in Ras family proteins during synaptic plasticity also revealed dynamic processes (Figure 4). The fact that Ras family proteins are highly dynamic may allow them to integrate signals across multiple learning experiences, and determine the optimal spacing between learning trials during incremental learning. In support of this idea, Pagani et al. (2009) and Philips et al. (2007) have recently found that the dynamic profiles of ERK activity, a canonical downstream element of Ras family proteins, significantly constrain the temporal windows between learning trials that are permissive for memory formation.

Finally, there are extensive functional interactions between different members of Ras family proteins as well as between Ras family proteins and other small G proteins in controlling cellular and molecular processes during memory formation (Figure 4C). These interactions regulate each step in memory processing, providing multiple levels of quality control to achieve the optimal signaling and functional outcome in response to different forms of extracellular stimuli.

For future studies, an important next step will be to generate conditional transgenic mice of Ras family-related proteins. Most studies on Ras family proteins in memory formation to
date have not been performed on conditional transgenic mice. These studies are subject to many caveats, such as lack of regional specificity, postnatal developmental effects, and global alterations in gene expression (Bilbo and Nelson, 2001; Crawley, 1999; Crawley andaylor, 1997). The generation of conditional transgenic mice expressing Ras family-related proteins under spatial and temporal control will greatly advance our understanding of the specific role of Ras family proteins in memory formation. It will also be important to cross different transgenic mice targeting distinct elements in the signaling of Ras family proteins to examine the interaction between multiple signaling elements in the network. Finally, it will be very informative to examine Ras family proteins in specific subcellular compartments within a restricted temporal window, in combination with careful behavioral designs, to evaluate the specific intracellular sites within neurons to which Ras family proteins are recruited during different stages of memory processing.

In conclusion, understanding how Ras family proteins contribute to neuronal plasticity and memory formation is not only of fundamental importance from a basic scientific perspective, but also from a clinical perspective. Genetic alterations in the signaling of Ras family proteins are closely linked to cognitive disorders associated with Alzheimer disease, autism, fragile X syndrome, and NF1, to name a few, which collectively affect one in a thousand people (Denayer et al., 2008b; Krab et al., 2008; Stornetta and Zhu, 2010). Importantly, recent studies suggest that these cognitive impairments can be at least partially reversed by readjusting related neuronal signaling elements (Ehringer et al., 2008; Li et al., 2005). Thus, the study of Ras family proteins in neuronal plasticity could significantly facilitate the development of effective therapies that could mitigate the cognitive impairments associated with these diverse diseases.

ACKNOWLEDGMENTS

We thank all members of the Carew laboratory for their helpful comments. This work is supported by National Institutes of Health Grant RO1 MH 041083 and National Science Foundation Grant IOB-0444762 (T.J.C.), and NIH Grant RO1 MH 081151 (T.J.C. and Kelsey Martin).

REFERENCES


Chang, L., Alicata, D., Ernst, T., and Volkow, N. (2007). Structural and metabo-
llic brain changes in the striatum associated with methamphetamine abuse.
Addiction 102 (Suppl 1), 16–32.

at excitatory synapses produced by direct activation of adenylyl cyclase in


Ras-GTPase activating protein (p125SynGAP) inhibited by CaM kinase II.
Neuron 20, 895–904.

P13 kinase signaling is required for retrieval and extinction of contextual

Forebrain-specific knockout of B-raf kinase leads to deficits in hippocampal

66, 273–280.

signaling is involved in Abeta-induced memory loss in Drosophila. Proc.
Natl. Acad. Sci. USA 107, 7060–7065.

growth factor beta1 alters synapsin distribution and modulates synaptic

Chiu, V.K., Bivona, T., Hach, A., SaJous, J.B., Silletti, J., Wiener, H., Johnson,

Cingolani, L.A., Thalhammer, A., Yu, L.M., Catalano, M., Ramos, T., Colicos,

onizes Ras-dependent activation of ERK1 and ERK2 by LPA and EGF in Rat-1
fibroblasts. EMBO J. 12, 3475–3485.

Costa, R.M., Federov, N.B., Kogan, J.H., Murphy, G.G., Stern, J., Ohno, M.,

Crawley, J.N. (1999). Behavioral phenotyping of transgenic and knockout
mice: experimental design and evaluation of general health, sensory func-

Crawley, J.N., and Paylor, R. (1997). A proposed test battery and constella-
tions of specific behavioral paradigms to investigate the behavioral pheno-
type of transgenic and knockout mice. Horm. Behav. 31, 197–211.

Cui, Y., Costa, R.M., Yang, T., Huynh, D.P., Pulst, S.M., Viskochil, D.H., Silva, A.J.,
and Brannan, C.I. (2001). Learning deficits, but normal development and tumor

Cui, Y., Costa, R.M., Giese, K.P., Elgersma, Y., Zhu, Y., Gutmann, D.H.,
O’Dell, T.J., and Colicos, M.A. (2006). Spred1 is required for synaptic plasticity and hippocampus-


Springer).

O’Dell, T.J., and Colicos, M.A. (2003). The RAS effector RIN1 modulates the

of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for

Eckelt-Mahan, K.L., Phan, T., Han, S., Wang, H., Chan, G.C., Scheiner, Z.S.,
11, 1074–1082.

Ehninger, D., Han, S., Shilyansky, C., Zhou, Y., Li, W., Kwiatkowski, D.J.,

Eickholt, B.J., Ahmed, A.I., Davies, M., Papakonstanti, E.A., Pearce, W.,
Control of axonal growth and regeneration of sensory neurons by the

Engelhardt, C.M., Bendschus, K., Messerschmitt, M., Renné, T., Walter, U.,
of Spred proteins in mouse and human tissues. Histochem. Cell Biol. 122,
527–533.


Farnsworth, C.L., Freshney, N.W., Rosen, L.B., Ghosh, A., Greenberg, M.E.,
exchange factor Ras-GRF. Nature 376, 524–527.

Fasan, S., and Brambilla, R. (2002). Cellular mechanisms of striatum-depen-

Fasan, S., D’Antoni, A., Orban, P.C., Valjent, E., Putignano, E., Varo, H.,
nucleotide-releasing factor 1 (Ras-GRF1) controls activation of extracellular
signal-regulated kinase (ERK) signalling in the striatum and long-term behav-


but not HRas in hippocampal neurons regulated by Ca2+/calmodulin. J. Cell
Biol. 170, 429–441.

Garcia, A.M., Rowley, M., Ackerman, K., Kowalczyk, J.J., and Lewis, M.D.
(1993). Peptidomimetic inhibitors of Ras farnesylation and function in whole

Gärtner, U., Alpár, Á., Reimann, F., Seeger, G., Heumann, R., and Arendt, T.
77, 630–641.

Enhanced Ras activity in pyramidal neurons induces cellular hypertrophy and changes in afferent and intrinsic connectivity in synRas mice. Int.

neurotransmitter release at excitatory central synapses. J. Neurosci. 28,
7991–8002.

P.V. (2008). Activation of exchange protein activated by cyclic-AMP enhances

long-lasting synaptic potentiation in the hippocampus. Learn. Mem. 15, 403–411.


J. Neurosci. 22
Koh, Y.H., Ruiz-Canada, C., Gorczyca, M., and Budnik, V. (2002). The Ras1-
mitogen-activated protein kinase signal transduction pathway regulates
Koh, N.E., Omer, C.A., Conner, M.W., Anthony, N.J., Davide, J.P., deSolms,
Kushner, S.A., Elgersma, Y., Murphy, G.G., Jaarsma, D., van Woerden, G.M.,
Post-translational modifications and regulation of the Ras superfamily of
GTPases as anticancer targets. Nat. Rev. Drug Discov. 6, 541–555.
Krabl, C., Goorden, S.M., and Elgersma, Y. (2008). Oncogenes on my mind:
Krapivinsky, G., Medina, I., Krapivinsky, L., Gapon, S., and Clapham, D.E.
(2004). SynGAP-MUPP1-CalMkkI synaptic complexes regulate p38 MAP
kinase activity and NMDA receptor-dependent synaptic AMPA receptor
potentiation. Neuron 43, 563–574.
Kusher, S.A., Elgersma, Y., Murphy, G.G., Jaarsma, D., van Woerden, G.M.,
Modulation of presynaptic plasticity and learning by the H-ras/extracellular
signal-regulated kinase/synapsin I signaling pathway. J. Neurosci. 25,
9721–9734.
Lambert, J.M., Lambert, Q.T., Reuther, G.W., Malliri, A., Sidorovsky, D.P.,
Sondek, J., Collard, J.G., and Der, C.J. (2002). Tiam1 mediates Ras activation
Li, W., Cui, Y., Kusher, S.A., Brown, R.A., Jentsch, J.D., Frankland, P.W.,
lovastatin reverses the learning and attention deficits in a mouse model of
guanine nucleotide-releasing factor 1 (Ras-GRF1) and Ras-GRF2 in the induc-
tion of long-term potentiation and long-term depression. J. Neurosci. 26,
1721–1729.
genetics in controlling the contribution of MAP kinases to synaptic plasticity.
Lin, C.H., Yeh, S.H., Lin, C.H., Lu, K.T., Leu, T.H., Chang, W.C., and Gean,
and synaptic plasticity in the amygdala. Neuron 37, 841–851.
of cell fate determination by p21ras/Ras1, an essential component of torso
Ma, N., Abel, T., and Hernandez, P.J. (2009). Exchange protein activated by
cAMP enhances long-term memory formation independent of protein kinase
Machida, N., Umikawa, M., Takei, K., Sakina, N., Myagmar, B.E., Taira, K.,
kinase kinase kinase 4 as a putative effector of Rap2 to activate the c-Jun
N-terminal kinase. J. Biol. Chem. 279, 15711–15714.
Manabe, T., Aiba, A., Yamada, A., Ichise, T., Sakagami, H., Kondo, H., and
Katsuki, M. (2000). Regulation of long-term potentiation by H-Ras through
Maruta, H., and Burgess, A.W. (1996). Regulation of the RAS Signaling
Matalanis, D., Sanz-Moreno, V., Arozarena, I., Calvo, F., Agudo-Ibáñez, L.,
Santos, E., Berciano, M.T., and Crespo, P. (2006). Distinct utilization of effec-
tors and biological outcomes resulting from site-specific Ras activation: Ras
functions in lipid rafts and Golgi complex are dispensable for proliferation
Protein tyrosine phosphatase SHP-2: a proto- oncogene product that
promotes Ras activation. Cancer Sci. 100, 1786–1793.
long-term synaptic facilitation by spaced and massed applications of sero-
tonin to sensory neurones synapses of Aplysia californica. Learn. Mem. 5,
246–256.
Merino, S.M., and Maren, S. (2006). Hitting Ras where it counts: Ras antago-
nism in the basolateral amygdala inhibits long-term fear memory. Eur.
J. Neurosci. 23, 196–204.
Mizuno, M., Yamada, K., Takei, N., Tran, M.H., He, J., Nakajima, A., Nawa, H.,
Morozov, A., Muzzio, I.A., Bourtchouladze, R., Van-Strien, N., Lapidus, K., Yin,
couples cAMP signaling to a distinct pool of p42/44MAPK regulating excit-
Mozzachioli, R., and Byrne, J.J. (2010). More than synaptic plasticity: role of
Muhia, M., Feldon, J., Knueseil, L., and Yee, B.K. (2009). Appetitively moti-
vised instrumental learning in SynGAP heterozygous knockout mice. Behav.
Neurosci. 123, 1114–1128.
heterozygote constitutive deletion of SynGAP. Eur. J. Neurosci. 31,
529–543.
9, 65–75.
GTPases, dendritic structure, and mental retardation. J. Neurobiol. 64, 58–74.
expression and astroglia in neurofibromatosis (type 1) brains. J. Neuropa-
thol. Exp. Neurol. 54, 586–600.
North, K.N., Riccardi, V., Samango-Sprouse, C., Ferner, R., Moore, B., Legius,
performance in neurofibromatosis 1: consensus statement from the NF1
North, K., Hyman, S., and Barton, B. (2002). Cognitive deficits in neurofibroma-
potentiation in the CA1 region of rat hippocampus via modulation of GTPase
signalling or inhibition of rho kinase. Neuropsycharmacology 46, 879–887.


involved in cell stretching modulation of p38 but not ERK or JNK MAP kinase. J. Cell Sci. 114, 1221–1227.


