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Neuronal RhoGEFs in synaptic physiology and behavior

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Abstract

In the mammalian brain the majority of excitatory synapses are housed in micron-sized dendritic protrusions called spines, which can undergo rapid changes in shape and number in response to increased or decreased synaptic activity. These dynamic alterations in dendritic spines require precise control of the actin cytoskeleton. Within spines, multidomain Rho guanine nucleotide exchange factors (Rho GEFs) coordinate activation of their target Rho GTPases by a variety of pathways. In this review, we focus on the handful of disease-related Rho GEFs (Kalirin; Trio; Tiam1; P-Rex1,2; RasGRF1,2; Collybistin) localized at synapses and known to affect electrophysiology, spine morphology, and animal behavior. The goal is to integrate structure/function studies with measurements of synaptic function and behavioral phenotypes in animal models.

Keywords

protein domain; LTP; receptor localization; knockout mouse

Introduction

Rho family GTPases play essential roles in regulating the complex adjustments to the actin cytoskeleton that underlie structural plasticity in dendritic spines, making the enzymes that regulate them a critical relay system for integrating synaptic activity and intracellular signaling. Rho guanine nucleotide exchange factors (Rho GEFs) catalyze GDP/GTP exchange while Rho GTPase activating proteins (Rho GAPs) accelerate GTP hydrolysis.

The synaptic Rho GEFs are multidomain proteins capable of responding to inputs from several signaling pathways and affecting an array of downstream targets. Proper expression and function of multiple Rho GEFs is necessary for spine formation and synaptic function. While their ability to activate Rho proteins with spatial and temporal precision is critical, the unique ability of the other domains to localize the protein, interact with specific ion channels and respond to intracellular signaling proteins makes the role of each Rho GEF distinct.

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A number of recent reviews provide comprehensive summaries of the many Rho GEFs with Dbl homology (DH) domains (Bouquier and others 2009; Kiraly and others 2010a; Mandela and Ma 2012; Penzes and others 2011; Penzes and Jones 2008; Rabiner and others 2005; Rossman and others 2005; Schiller 2006; Tolias and others 2011). We focus here on the handful of Rho GEFs known to affect behavior and synaptic function. Structurally related Rho GEFs are discussed together, with the goal of integrating structure/function studies with measurements of synaptic function and behavior.

Dual Rho GEFs (Kalirin and Trio)

Discovery and expression

Trio was first identified as an interactor with the cytosolic domain of LAR, a receptor protein tyrosine phosphatase localized to focal adhesions (Debant and others 1996). Kalirin was first identified as an interactor with the cytosolic domain of peptidylglycine α-amidating monooxygenase, a secretory granule membrane protein (Alam and others 1996). The proteins are 64% identical (rat Kalirin vs. rat Trio; AF232669.1 vs. XP_003753588.1) (McPherson and others 2002) and, discovered under different circumstances, would likely have been referred to as Trio- or Kalirin-1 and -2. Trio and Kalirin are notable for the presence of two Rho GEF domains of differing specificity along with a Ser/Thr kinase and multiple additional protein-protein interaction domains (Fig.1). A single common ancestor is found in *Drosophila* (*dTrio*) and in *C.elegans* (*unc-73*). The *Drosophila* protein is 42–43% identical to both rodent Kalirin and Trio; comparisons to Unc-73 also lead to the conclusion that *Kalrn* and *Trio* arose from duplication of a common ancestor.

Kalirin and Trio are both widely expressed in the brain, primarily localized to neurons at all stages of development (Ma and others 2005; Ma and others 2001; Peng and others 2010). Analysis of *dTrio* mutants highlighted its role in axon formation and pathfinding (Awasaki and others 2000; Bateman and others 2000; Liebl and others 2000) Trio is most importantly expressed in cerebellar granule cells, while Kalirin is most highly expressed in long projection neurons in the cortex and hippocampus, with less expression in the cerebellum and brainstem. The developmental expression patterns of both genes are isoform- and region-specific. Expression of *Kalrn* and *Trio* is regulated independently; for example, compensatory increases in *Trio* expression have not been observed in *Kalrn* knockout mice (Ma and others 2008b).

Importantly, both genes are also expressed outside of the nervous system. Kalirin, primarily the Kal9 isoform (Fig.1A), is expressed early in development in a wide variety of tissues throughout the body (Hansel and others 2001), primarily lung, muscle, GI epithelium and pancreas. In the adult, Kal7 is the major form in mature neurons, while Kal9 and Kal12 are found at lower levels in aorta, skeletal muscle and endocrine tissues. Kal7 is primarily expressed in postsynaptic structures such as dendritic spines, while Kal12 is especially elevated in growth cones (Xin and others 2009) (Fig.2A). Expression of Kal7 correlates with the onset of synaptogenesis in the cortex.

Protein structure and signaling

Both genes undergo extensive alternative splicing, yielding functionally distinct isoforms ranging from about 100 kDa to nearly 400 kDa in size (Fig.1). For both Kalirin and Trio, GEF1 activates Rac1 and RhoG, while GEF2 activates RhoA; splice variants that include only one or the other GEF domain have been identified. Notably, the kinase domain encoded by *Kalrn* and *Trio* is absent from *dTrio* and *unc-73.* Both the Sec14 domain of Kalirin, which binds specific phosphoinositides, and its nine spectrin repeats play essential roles in its function (Schiller and others 2008). The spectrin repeat region, SH3 domains and Ig/FN

domains of Kalirin and Trio interact with a wide variety of proteins including iNOS, DISC1, Arf6, afadin, PAM, and HAP1.

Recombinant Kal7 is phosphorylated by several of the protein kinases concentrated at the PSD; consistent with this, Kal7 isolated from rat brain is heavily phosphorylated (Kiraly and others 2011b). Several sites in Kalirin are targeted by multiple protein kinases (e.g. protein kinase A, protein kinase C, CaMKII and CKII), leading to the suggestion that Kalirin participates in signaling pathways initiated by a variety of pathways (Kiraly and others 2011b; Penzes and others 2003).

Detailed examination of mutations in *unc-73* established a role for its first GEF domain in neuronal development (Williams and others 2007) and a role for its second GEF domain in adult neuronal function (Chan and others 2012; McMullan and others 2012; Williams and others 2007). In *D.melanogaster*, a similar picture emerges; the N-terminal half of *dTrio*, which includes GEF1, is crucial to neurite outgrowth (Awasaki and others 2000; Bateman and others 2000; Luo 2000), while neuromuscular junction establishment and function require the GEF2 region (Astigarraga and others 2010; Ball and others 2010). Liprin, a cytoplasmic binding partner of LAR, is thought to act through *dTrio* to promote correct targeting of axons, while retrograde BMP signaling from muscle to the nerve terminal acts through *dTrio* to regulate synapse development (Astigarraga and others 2010; Ball and others 2010). Expression of BMP in muscle increases dTrio expression in motorneurons quite dramatically (Ball and others 2010).

The GEF2 domain of Trio and Kalirin shares with p63RhoGEF the ability to bind to and be activated by $G\alpha_q$, placing these RhoGEFs downstream of selected G protein coupled receptors (Lutz and others 2007; Rojas and others 2007). Trio and $G\alpha_q$ co-immunoprecipitate when co-expressed in hEK293 cells (Williams and others 2007). Acetylcholine acting through a muscarinic receptor to activate $G\alpha_q$ results in increased sphingosine kinase activation and increased levels of sphingosine-1-P in the presynaptic terminal, potentially activating Trio or Kalirin GEF2 at the same time and increasing neurotransmitter release (Chan and others 2012; McMullan and others 2012; Williams and others 2007).

Behavior

The $Trio^{-/-}$ mouse dies in late gestation (O'Brien and others 2000) with spherical myofibers and major defects in secondary myogenesis. Subsequent work (Briancon-Marjollet and others 2008) revealed defective neuronal outgrowth, complete lack of the anterior commissure and defects in the internal capsule and corpus callosum. These defects reflect loss of the interaction of Trio with the netrin-1 receptor, Deleted in Colon Cancer (DCC), which is essential for normal Rac1 activation in axonal projections. Because of the lethality of the global $Trio^{-/-}$ knockout, cell-type specific knockouts using Cre recombinase expression driven by the nestin or GFAP promoter were created (Peng and others 2010). Only 10% of $Trio^{nestin\text{-}KO}$ mice survived for a day; they lacked a cerebellum, were severely ataxic and all died within 3 weeks without ever gaining significant weight. In contrast, $Trio^{GFAP\text{-}KO}$ mice appeared unaffected. Southern blot analysis demonstrated complete excision of the floxed allele in brain tissue from $Trio^{nestin\text{-}KO}$ mice (Peng and others 2010), consistent with early expression of nestin-Cre and elimination of Trio expression in glial, as well as neuronal, lineages.

Knockout of one copy of *dTrio* results in >50% decrease in Rac1-dependent synaptic bouton numbers (Ball and others 2010). Rac1 overexpression increases the quantal content of neuromuscular junction excitatory junction potentials (EJP), with no change in the unitary size of the quanta (Ball and others 2010) (Fig.2B). Likewise *dTrio* knockout decreased the

quantal content of EJPs, and the EJP enhancement seen with Rac1 overexpression was largely blocked in the *dTrio* knockout. Interestingly, bone morphogenic protein (BMP) from the muscle is required for normal *dTrio* expression in the innervating neuron, and decreasing *dTrio* expression directly (as in *trio*/+ flies) or indirectly (BMP lowered) results in reduced neuromuscular junction growth; expression of *dTrio* in motorneurons, but not in the muscle, reverses the deficit. The interpretation is that the level of expression of neuronal *dTrio*, and consequent activation of Rac1, directly correlates with the size of the presynaptic terminal and thus the quantal content of the EJPs (Ball and others 2010).

Two approaches were used to generate mouse models lacking all of the major *Kalrn* isoforms (KalGEF1^{KO} and KalSR^{KO}) (Mandela and others 2012; Xie and others 2011) and Kal7 was specifically targeted in another model (Kal7^{KO}) (Ma and others 2008b); each yielded viable homozygous knockout mice. In KalGEF1^{KO} and Kal7^{KO} mice, the density of hippocampal dendritic spines is decreased compared to normal (Ma and others 2008b; Xie and others 2011). Cortical cultures prepared from Kal7^{KO} mice showed almost a 50% decrease in synaptic density; in addition, Vglut1-positive puncta that were not apposed to a PSD-95 positive postsynaptic ending were observed commonly in Kal7^{KO} cultures but only rarely in wildtype neurons, suggesting a role for Kal7 in synaptic maturation (Ma and others 2008b).

Although lack of Rac1 and RhoG activation in the Kal7^{KO} neurons plays a major role (May and others 2002), loss of other protein-protein interactions is also of importance. The first PH domain of Kalirin interacts with the juxtamembrane region connecting the final transmembrane domain of the NR2B subunit of the NMDA receptor with its cytosolic domain (Kiraly and others 2011a). Loss of this interaction presumably contributes to the decrease in NR2B-containing NMDA receptors observed in PSDs isolated from Kal7^{KO} mice (Kiraly and others 2011a; Lemtiri-Chlieh and others 2011). Genotypic differences in several of the behavioral deficits observed in Kal7^{KO} mice (conditioned place preference for cocaine and passive avoidance fear conditioning) were largely abrogated by pretreatment with ifenprodil, a selective blocker of NR2B-containing NMDA receptors, confirming the importance of the Kalirin/NR2B interaction (Kiraly and others 2011a). Kal7^{KO} mice show enhanced locomotor sensitization to cocaine administration (Kiraly and others 2010b).

A striking attribute of both the Kal7^{KO} and the KalSR^{KO} mice is the decrease in anxiety-like behavior on the elevated zero maze, along with an inability to acquire a fear-based passive avoidance task normally (Ma and others 2008b; Mandela and others 2012). Similarly, context and cued fear-based conditioning was impaired in the KalGEF1^{KO} mouse (Xie and others 2011). Deficits in rotarod performance and in the neuromuscular junction were observed in KalSR^{KO} mice, but not in Kal7^{KO} mice; a role for Kalirin/Trio at the neuromuscular junction is consistent with observations made in *Drosophila*. Analysis of KalSR$^{KO/+}$ mice revealed a role for Kal9 in the activation of Rac1 in smooth muscle cells and in smooth muscle cell migration and proliferation (Wu and others 2012).

Electrophysiology and synaptic function

When hippocampal LTP was recorded using a theta burst pattern paired with a depolarizing pulse in the clamped cell, pyramidal neurons in acute slices from Kal7^{KO} animals showed less than half as much potentiation as seen using wildtype slices (Fig.2C) (Ma and others 2008b). Further studies established that the NMDA/AMPA current ratio in Kal7^{KO} mice was depressed to half in layer 2/3 cortical neurons, compared to the value in wildtype mice, which almost guarantees the LTP deficit seen in the Kal7^{KO} mice (Kiraly and others 2011a). The decreased amount of NR2B subunit present at the PSD and on the cell surface is presumably a direct result of the loss of Kalirin PH domain binding to the juxtamembrane domain of the NR2B subunit (Kiraly and others 2011a). A similar diminution in LTP was

observed in KalGEF^{KO} mice vs. wildtype mice, using a high frequency stimulation paradigm to induce LTP (Xie and others 2011). Additional electrophysiological studies demonstrated that non-NMDA receptor-dependent LTP was normal in Kal7^{KO} mice, while NMDA receptor-dependent LTD was abolished (Fig.2C) (Lemtiri-Chlieh and others 2011), focusing attention on NMDA receptor related deficiencies. Bath application of NPPD, a Kalirin/Trio GEF1-selective blocker, greatly decreased LTP as did the loss of the Kal7 protein, implicating the GEF1 domain in the electrophysiological changes seen in Kal7^{KO} mice (Lemtiri-Chlieh and others 2011). The decreased paired pulse ratio (2nd evoked EPSC not as much larger than the closely paired 1st evoked EPSC as in wildtype) observed in KalGEF1^{KO} mice (Xie and others 2011) may reflect decreased levels of Kal9 and Kal12. Kal9 and Kal12 are known to occur in presynaptic terminals and growth cones (Xin and others 2009), and their loss may underlie some of the hormone secretion abnormalities observed in the KalSR^{KO} mice (Mandela and others 2012).

The Kal7^{KO} mouse was studied extensively, using both hippocampal and cortical slices. Correlating with a 15% decrease in dendritic spine density, recordings from hippocampal slices showed a 50% decrease in sEPSC frequency with no change in sEPSC amplitude, as might be predicted if not all of the remaining dendritic spines are fully functional (Ma and others 2008b). Similarly, the mEPSP frequency was significantly lower in frontal cortex slices from KalGEF1^{KO} mice, again with normal amplitude (Cahill and others 2009).

Disease relevance

Mutations at the *KALRN* locus have been strongly associated with schizophrenia (Kushima and others 2012) and early onset coronary artery disease (Wang and others 2007). Loss of one copy of *Kalrn* produces mice with arterial smooth muscle cells which show reduced proliferation, migration and reduced Rac1 activation after carotid endothelial denudation (Wu 2012). Alzheimer's patients have significantly less Kal7 in hippocampal extracts than non-demented patients (Youn and others 2007). Kal7 mRNA and protein are increased in both rats and mice in response to cocaine (Kiraly and others 2010b; Ma and others 2012; Mains and others 2011); since Kal7 over-expression leads to increased dendritic spines in a number of neuronal systems (Ma and others 2008c), it is not surprising that Kal7^{KO} mice do not exhibit the usual increase in nucleus accumbens dendritic spine numbers in response to chronic cocaine (Kiraly and others 2010b). Chronic prolonged restraint stress has the opposite effect, decreasing the level of Kal7 in the hippocampus to half the normal level (Li and others 2010).

T-cell lymphoma invasion and metastasis family (Tiam1 and Tiam2)

Discovery and expression

Tiam1 was identified as a T-lymphoma invasion and metastasis-inducing gene by retroviral insertional mutagenesis and encodes a GEF specific for Rac (Habets and others 1994; Michiels and others 1995). Tiam1 is widely expressed in the developing central nervous system, and expression continues at postnatal stages (E14.5 to P180), with an established role in neuronal migration and axonal extension both *in vitro* and *in vivo* (Ehler and others 1997; Kawauchi and others 2003). Subcellularly, Tiam1 is localized to dendrites and dendritic spines, where it is enriched at the PSD (Tolias and others 2005). Tiam2, a closely related gene, is widely expressed in both in mouse and human brain (Chiu and others 1999).

Due to its roles outside of the CNS, Tiam1 has been extensively studied in cancer research and T-cell migration (Gerard and others 2009; Malliri and others 2002). A recent study demonstrated that Tiam1-Rac1 signaling antagonizes centrosome separation during prophase and is required to balance Kinesin-5-induced forces during bipolar spindle

assembly (Woodcock and others 2010). These results suggest that Tiam1 controls cell division, and the disruption of Tiam1 in neuroepithelial cellssupports the observation of severe brain abnormalities in one Tiam−/− mouse line (Yoo and others 2012).

Protein structure and function

Tiam1 is a multi-domain protein with a myristoylation site at its N-terminus; two PEST sequences, a PH-CC-Ex domain (a PH domain, a putative coiled-coil region, and a conserved extra region), a Ras-binding domain and a PDZ domain precede the catalytic DH domain, which is followed immediately by a PH domain and a PDZ binding motif at the C-terminus (Fig.3A). Tiam2, also referred to as STEF (SIF and Tiam1 like-exchange factor), lacks a myristoylation signal and PEST sequences but otherwise shares significant homology to Tiam1; portions of Tiam1 and Tiam2 are 70% identical (Chiu and others 1999; Hoshino and others 1999). Knocking down Tiam1 and Tiam2 using antisense oligonucleotides or shRNAs affects neurite length and morphology (Goto and others 2011; Kunda and others 2001), suggesting that both proteins contribute to neurite outgrowth; we focus on Tiam1 because it is necessary for dendritic arbor and spine formation.

Behavior

Tiam1 knockout mouse lines were created by Malliri et al. (Malliri and others 2002) and by Yoo et al.(Yoo and others 2012). Tiam1$^{-/-}$ mice generated by ablating exon 2 grow and reproduce normally (Malliri and others 2002); these mice are primarily used in cancer biology and immunology research. The other Tiam1 knockout mouse line was generated using a Tiam1 gene-trapped embryonic stem cell line, which targets the intron separating exons 5 and 6, potentially creating a truncated Tiam-β-galactosidase fusion protein (Yoo and others 2012). Although no other genes are known to lie in the targeted region, these E9.5 and E10.5 Tiam$^{-/-}$ embryos exhibit anencephaly and exencephaly and do not survive beyond E13.5, indicating that Tiam1 is important for early forebrain development (Yoo and others 2012). Despite high expression levels in the brain and an obvious role in CNS function, to our knowledge there have been no behavioral or electrophysiological studies performed with homozygous or heterozygous Tiam1$^{-/-}$ animals.

Electrophysiology and synaptic function

RNAi-mediated knockdown of Tiam1 in rat hippocampal neurons dramatically reduces dendritic arborization and spine density (Tolias and others 2005; Tolias and others 2007; Zhang and Macara 2006) as well as frequency of spontaneous miniature excitatory postsynaptic currents (mEPSC) (Fig.3B). Decreasing Tiam1 inhibits neuronal migration and prevents axon formation, with most neurons exhibiting smaller growth cone size and abnormal axon cytoskeletal organization (Kunda and others 2001; Matsuo and others 2003) as well as neuronal migration (Kawauchi and others 2003). MAP1B interacts directly with Tiam1 (Fig.4), and over-expression of Tiam1 rescues the axonal growth defects observed in MAP1B-deficient neurons. These data suggest that the MAP1-Tiam1 interaction plays an important role in Rac1 regulation and is required for axonal elongation (Fig.4) (Montenegro-Venegas and others 2010).

Tiam1 over-expression induces extension of multiple axon-like neurites (Kunda and others 2001; Matsuo and others 2003), and also results in increased dendritic spine density (Tolias and others 2005), further suggesting a roles in spine and axon formation. Tiam1 interacts directly with the NR1, but not any of the NR2 subunits of the NMDA receptor, and is phosphorylated in a Ca^{2+}-dependent manner following NMDA receptor stimulation. Knocking down Tiam1 expression in rat cortical neurons blocks NMDA receptor-induced increases in dendritic spine density, indicating that Tiam1-dependent Rac1 activation is required for NMDA receptor-mediated spine formation (Tolias and others 2005).

The PH-CC-Ex domain of Tiam1 interacts with the EphB2 receptor tyrosine kinase in a kinase-dependent manner. RNAi-mediated knockdown of Tiam1 expression blocks the ability of ephrin B1 to increase dendritic spine density in rat hippocampal neurons (Tolias and others 2007). Interestingly, the Tiam1 PH-CC-Ex region acts in a dominant-negative manner, probably by binding to Tiam1 interacting proteins and preventing recruitment of endogenous Tiam1 to the membrane (Fig.4) (Tolias and others 2005; Tolias and others 2007). The crystal structure of PH-CC-Ex reveals that this region is a novel protein- and membrane-binding module, strongly binding the NMDA receptor loop I peptide (residues 591–610) and not the C-terminal tail peptide (residues 921–938) (Fig. 4) (Terawaki and others 2010). This type of interaction is reminiscent of the binding of Kalirin-PH1 to the juxtamembrane domain of the cytoplasmic tail of NR2B (Kiraly and others 2011a).

Tiam1 also binds to partitioning-defective gene 3 (PAR-3), a member of the PAR polarity complex, which contributes to spine morphogenesis (Zhang and Macara 2006). PAR-3 spatially restricts Tiam1 to dendritic spines, contributing to proper spine formation (Fig. 4) (Zhang and Macara 2006). Finally, Tiam1 is involved in the BDNF-TrkB signaling pathway. Tiam1 is required for BDNF-induced spine morphogenesis and neurite extension; BDNF-activated TrkB directly binds to and activates Tiam1 by phosphorylating Tyr^{829}, and a point mutation at this residue blocks BDNF-induced morphological changes (Lai and others 2012; Miyamoto and others 2006). In maturing postnatal cerebellar granule cells, BDNF up-regulated expression of Tiam1 and the NR2C NMDA receptor via the TrkB-Erk cascade. This up-regulation is blocked not only by inhibition of signaling pathways, but also by suppression of the Etv1/Er81 transcription factor using Etv1 siRNA (Fig. 4) (Abe and others 2012).

Disease relevance

In hippocampal neurons, Tiam1 is activated and recruited to membranes following exposure to amyloid beta peptide (Aβ), and thus may be involved in Alzheimer's disease pathology (Ma and others 2008a; Mendoza-Naranjo and others 2007). In addition to the roles of Tiam1 in the nervous system, $Tiam1^{-/-}$ mice are resistant to Ras-induced skin tumors, suggesting that Tiam1 is a critical regulator of tumor formation and invasion (Malliri and others 2002; Vigil and others 2010).

Phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac Exchange Factor Family (P-Rex1 and P-Rex2)

Discovery and expression

Two gene family members have been characterized in human (*PREX1*, *PREX2*) and mouse (*Prex1*, *Prex2*) (Fig.5A). P-Rex1 was purified from neutrophil cytosol on the basis of its phosphatidylinositol(3,4,5)trisphosphate [$PI(3,4,5)P_3$]-sensitive Rac GEF activity (Welch and others 2002); Rac activation in these cells plays an essential role in chemotaxis, phagocytosis and reactive oxygen species (ROS) formation. The Rac GEF activity of purified P-Rex1 was also shown to be stimulated by the addition of recombinant $G\beta_1\gamma_2$ (Welch and others 2002; Hill and others 2005). The effects of $PI(3,4,5)P_3$ and $G\beta\gamma$ on Rac activation are synergistic, leading to the concept that P-Rex1 could serve as a coincidence detector for the simultaneous activation of both signaling pathways. P-Rex2, which was identified by homology, is 59% identical to P-Rex1. As for P-Rex1, $PI(3,4,5)P_3$ and $G\beta\gamma$ exert a synergistic effect on the ability of P-Rex2 to activate Rac1 (Donald and others 2004).

Northern blot analysis of human tissue reveals high levels of P-Rex1 mRNA in neutrophils, with slightly lower levels in brain (Donald and others 2008; Welch and others 2002). P-Rex1 mRNA expression in brain is readily detected from E13 through adulthood, with levels

highest from E17 through P7 (Yoshizawa and others 2005). Based on Western blot analysis, P-Rex1 expression is widespread, but highest in the cerebellum (Donald and others 2008). *In situ* hybridization reveals a complex and rapidly changing pattern for P-Rex1, with expression in cerebral cortex, trigeminal ganglion and dorsal root ganglia readily apparent at E12 (Donald and others 2008). In the adult, expression of P-Rex1 is most notable in layers II/III of the cortex and in the Purkinje cell layer of the cerebellum. In tissue sections and cultured neurons, endogenous P-Rex1 protein localizes to the shafts of neurites (Yoshizawa and others 2005). As hippocampal neurons differentiate in culture, P-Rex1 localizes to the distal part of the axon and axonal growth cones; expression of exogenous P-Rex1 enlarges axonal, but not dendritic, growth cones (Waters and others 2008).

P-Rex2 mRNA is most highly expressed in heart, skeletal muscle and placenta, but is also present in adult brain (Donald and others 2004). Western blots reveal similar levels of P-Rex2 in brain and lung (Donald and others 2008); *in situ* hybridization and immunostaining identify cerebellar Purkinje cells as the major site of P-Rex2 expression in adult brain, with lower levels in frontal cortex, striatum, amygdala and hippocampus.

Protein structure and function

The catalytic Dbl homology domain of P-Rex occurs near its N-terminus, followed by a PH domain, two DEP (Disheveled, EGL-10, Pleckstrin) domains, two PDZ domains and an inositol polyphosphate 4-phosphatase-like domain (Fig.5A). $PI(3,4,5)P_3$ binds to the PH domain while Gβγ subunits interact with the DH domain (Fig.5B); binding of either $PI(3,4,5)P_3$ or Gβγ stimulates the ability of P-Rex to activate Rac, with a synergistic effect of the two activators. P-Rex1 activates Rac1 and Rac3 (Rac1B), a neuron-specific Rho GTPase (Waters and others 2008).

Intact P-Rex1 is 60-fold less active than its isolated DH/PH domain; the DEP and PDZ domains play a role in keeping P-Rex1 inactive. $PI(3,4,5)P_3$ increases the activity of the isolated DH/PH domain of P-Rex1, but has a far greater effect on the activity of full-length P-Rex1. Gβγ seems to require only the DH domain to activate P-Rex1; as for $PI(3,4,5)P_3$, the effect of Gβγ on Rac activation is substantial (Hill and others 2005). The ability of the Gβγ dimer released upon GPCR stimulation to activate P-Rex1 depends on its Gγ subunit (Mayeenuddin and others 2006). A yeast two-hybrid screen using the tandem DEP domains of P-Rex1 and a human brain cDNA library identified the C-terminal region of mammalian target of rapamycin (mTOR), including its serine/threonine kinase domain, as an interactor (Fig.5B) and both P-Rex1 and P-Rex2 have been identified as components of the mTORC2 complex (Hernandez-Negrete and others 2007). Phosphorylation of P-Rex1 by PKA alters its intramolecular interactions and prevents its binding and activation by Gβγ (Urano and others 2008).

The activation of Rac1 and Rac2 in neutrophils triggers a variety of responses including chemotaxis, generation of ROS and secretion of azurophilic granules. It is striking that the absence of P-Rex1 has quite distinct effects on these responses (Welch and others 2005). The ability of unprimed or tumor necrosis factor α-primed P-Rex1$^{-/-}$ neutrophils to produce ROS in response to formyl-Met-Leu-Phe (fMLP) is only slightly diminished; in contrast, the ability fMLP to stimulate ROS production by lipopolysaccharide-primed neutrophils is almost entirely eliminated (Welch and others 2005).

The GEF activity of P-Rex1, which is expressed endogenously in PC12 pheochromocytoma cells, is activated by NGF, resulting in increased membrane ruffling and cell motility (Yoshizawa and others 2005). Along with the DH domain, the PDZ and phosphatase-like domains of P-Rex1 play essential roles in its ability to stimulate membrane ruffling. PC12 cell migration in response to NGF is greatly increased upon expression of exogenous P-

Rex1; the process is dependent on activation of Rac1. Deletion of the DH domain of P-Rex1 (ΔDH-P-Rex1) yielded a dominant negative variant of P-Rex1, which blocks NGF-induced PC12 cell migration.

Behavior

P-Rex1, P-Rex2 and P-Rex1/P-Rex2 knockout mice have been characterized. P-Rex1$^{-/-}$ mice are viable and healthy, perform normally in tests of motor behavior, and are slightly smaller than wildtype mice (Donald and others 2008; Welch and others 2005) P-Rex2$^{-/-}$ mice are viable and fertile although females have lower than normal body weight (Donald and others 2008). The brains of P-Rex2$^{-/-}$ mice show no major anatomical defects, but Purkinje cell dendritic structure is abnormal. Rotarod performance in adult female P-Rex2$^{-/-}$ mice is somewhat compromised, while male performance is less affected (Donald and others 2008). Basic sensory motor functions and gait are normal. P-Rex1$^{-/-}$/P-Rex2$^{-/-}$ mice are viable and fertile, with sex-specific changes in body weight. Brain anatomy is grossly normal, with disordered Purkinje cell dendrites, as observed in the P-Rex2$^{-/-}$ mouse brain. The motor behavior of male and female P-Rex1$^{-/-}$/P-Rex2$^{-/-}$ mice is more impaired than that of P-Rex2$^{-/-}$ mice, with substantial deficits in rotarod performance, open field behavior, beam walking and gait (Donald and others 2008). Deficits worsen with age and are more pronounced in females than in males.

Electrophysiology and synaptic function

The prevalence of P-Rex1 and P-Rex2 in cerebellar Purkinje cells, the altered Purkinje cell dendritic morphology and altered motor function observed in mice lacking both P-Rex proteins identified parallel fiber/Purkinje cell synapses as a likely site at which to observe alterations in synaptic transmission (Fig.5C). LTD at these synapses, which can be evoked by co-stimulation of parallel fiber and climbing fiber inputs, involves increased phosphorylation of AMPA receptor GluR2 subunits, which promotes their internalization and decreases the response to transmitter release (Jackson and others 2010). The parallel fiber-Purkinje synapse is essential for the eye-blink reflex.

LTP at these same synapses can be evoked by stimulating the parallel fibers alone; although less well understood than LTD, it is clear that LTP involves phosphatase-mediated reversal of GluR2 phosphorylation and decreased AMPA receptor internalization. Consistent with a role for P-Rex, inhibition of PI3K abolishes LTP entirely. Synthesis of NO is required for LTP at these synapses and NO donors can evoke LTP. P-Rex1 and P-Rex2 are activated by PI(3,4,5)P_3, the product of Class I PI3Ks, which are essential for the induction of hippocampal LTP and NMDA receptor evoked increased surface expression of GluR1-containing AMPA receptors in hippocampal neurons.

Sagittal slices from the cerebellar vermis of P21-P28 mice, were used (Jackson and others 2010); despite the altered dendritic morphology observed in P-Rex1$^{-/-}$/P-Rex2$^{-/-}$ mice, synaptic transmission, short-term plasticity and passive membrane properties were unaltered. LTP was induced at parallel fiber Purkinje cell synapses by stimulating at 1 Hz for 5 min. In wildtype mice, this triggers an increase in parallel fiber EPSC amplitude that lasts over 30 min; with no change in paired-pulse ratio, the effect is thought to be post-synaptic (Fig.5C, red). In the P-Rex1$^{-/-}$/P-Rex2$^{-/-}$ mice, EPSC amplitude shows an initial increase but this increase is not sustained (Fig.5C, **green**). Parallel fiber LTP can also be evoked by exogenous NO donors; the initial response of P-Rex-deficient slices to an exogenous NO donor resembles the wildtype response, but potentiation is not sustained. Hence, P-Rex family enzymes are required for the maintenance of cerebellar LTP.

Disease relevance

PREX1 maps to a Type 2 diabetes mellitus susceptibility locus. Insulin is known to activate PI3K, yielding $PI(3,4,5)P_3$ and muscle cells that lack Rac1 are known to exhibit impaired insulin-stimulated GLUT4-translocation. Although Rac1 does not promote GLUT4 translocation when insulin levels are saturating (100 nM), it does at physiological levels of insulin (1–10 nM). In the absence of insulin, expression of exogenous P-Rex1 in adipocytes has no effect on GLUT4 trafficking; however, the effect of insulin on GLUT4 trafficking is enhanced. Pre-treating adipocytes with a PI3K inhibitor decreases the ability of P-Rex1 to enhance the actions of insulin (Balamatsias and others 2011).

Ras-guanine nucleotide-releasing factor family (RasGRF1 and RasGRF2)

Discovery and expression

Although initially identified due to their sequence homology to yeast CDC25, a potent Ras GEF, the mammalian Ras-guanine nucleotide-releasing factors (RasGRFs) are large, multidomain proteins containing both Ras GEF and Rac GEF domains. *RASGRF1* (mouse chromosome 9, human 15) is paternally imprinted and expressed only after birth. *RASGRF2* (mouse chromosome 13, human 5) is not imprinted, but its expression is developmentally regulated; low early in development, expression increases around postnatal day 10 in the rat and remains high in adulthood. Both RasGRF1 and RasGRF2 are predominantly expressed in neurons of the adult brain, although RasGRF2 is also highly expressed in other tissues (Chen and others 1993; Feig 2011). In addition to the two full-length proteins, smaller alternatively spliced transcripts have been identified; some have unique developmental expression patterns and differential effects on downstream signaling, suggesting diverging physiological roles (see (Fernandez-Medarde and Santos 2011) for review). Our discussion here will focus on the full-length gene products, which have been studied in more detail.

Protein structure and function

Full length RasGRF proteins contain an N-terminal PH domain followed by a coiled coil domain, a Ca^{2+}/calmodulin-binding IQ domain, the tandem DH/PH domains common to Dbl family Rho GEFs, a Ras exchange motif (REM), and a Ras GEF domain (CDC25) (Fig. 6A). *In vitro* and *in vivo* studies have shown that both full-length RasGRFs are capable of activating Ras family members as well as the Rho GTPase Rac1. The IQ domain of RasGRF1 binds calmodulin in a Ca^{2+}-dependent manner, leading to increased GEF activity in cells (Farnsworth and others 1995), but not *in vitro* (Baouz and others 1997). A RasGRF2 mutant lacking the IQ domain altogether is still able to activate Ras, but does not result in activation of its downstream effector, Erk (de Hoog and others 2000). These results have not been entirely reconciled, but the hypothesis that RasGRF activity requires proper protein localization and assembly of downstream networks prevails. The N-terminal PH and CC domains may also be involved in protein localization. RasGRF1, but not RasGRF2, contains a “Neuronal domain (ND)”, which lets RasGRF1 associate with NMDA-type glutamate receptors through a direct interaction (Fig.6A); the ND interacts directly with the cytosolic tail of the NR2B subunit (Krapivinsky and others 2003) (Fig.7). Disruption of this interaction results in reduced Erk activation in cultured hippocampal neurons, while leaving NMDA receptor-mediated current intact following bath application of NMDA receptor agonists (Krapivinsky and others 2003).

A role for RasGRF1 has also been identified in Ca^{2+}-dependent signaling downstream of various GPCRs and receptor tyrosine kinases. PKA-mediated phosphorylation and activation of RasGRF1 is also necessary for Erk activation following stimulation of exogenous $G\alpha_s$-coupled $5\text{-}HT_7$ serotonin receptors in HEK-293 cells (Norum and others 2005). Stimulation of TrkA receptors, dopamine receptors and muscarinic acetylcholine

receptors also results in RasGRF1 activation. The mechanisms and consequences of these interactions have not been fully elucidated, but all appear to require RasGRF1 phosphorylation, most at Ser^{916}, as well as its Ca^{2+}-dependent association with calmodulin (Mattingly 1999; Mattingly and Macara 1996; Norum and others 2005; Yang and others 2003).

Behavior

The availability of several transgenic mouse lines has established the physiological significance of the RasGRF family. Phenotypically, mice lacking RasGRF1 ($RasGRF1^{-/-}$) are smaller than their wildtype littermates, and behavioral tests have revealed impaired fear conditioning in $RasGRF1^{-/-}$ mice. Normal performance on hippocampal memory tasks was seen in mice lacking both the full-length RasGRF1 and a less studied splice variant of RasGRF1, p55-GRF1; however numerous studies using mice lacking only the full-length RasGRF1 isoform have shown impaired hippocampal-dependent memory (Brambilla and others 1997; d'Isa and others 2011; Giese and others 2001; Li and others 2006). Locomotor sensitization and conditioned place preference for cocaine are significantly reduced in $RasGRF1^{-/-}$ mice, while both are exacerbated in animals over-expressing RasGRF1 (Fasano and others 2009). RasGRF1 may also be involved in CB1 (endocannabinoid) receptor sensitization and tolerance to Δ9-THC, since $RasGRF1^{-/-}$ mice have altered gene expression profiles following chronic Δ9-THC exposure (Rubino and others 2006). Visual phenotypes, including deficient photoreception, have also been observed and likely arise from RasGRF functions in the retina (Fernandez-Medarde and others 2009). $RasGRF2^{-/-}$ mice grow and reproduce normally (Fernandez-Medarde and others 2002) and most reported phenotypes of single and combined knockout strains have indicated RasGRF2 involvement in immune mechanisms.

Electrophysiology and Synaptic Function

Using theta burst stimulation, Brambilla et al. showed impaired LTP in the amygdala of $RasGRF1^{-/-}$ mice, though hippocampal LTP appeared normal (Brambilla and others 1997). They also observed elevated basal field activity in both amygdala and hippocampal slices (Brambilla and others 1997). Furthermore, recordings from cultured neurons and acute brain slices revealed hippocampal hyperexcitability in $RasGRF1^{-/-}$ mice, and convulsant drug administration studies have shown increased seizure susceptibility in these animals (Tonini and others 2001).

Although the RasGRF proteins are structurally similar (Fig.6A), diverging roles in synaptic function are apparent. Both RasGRF1 and RasGRF2 act downstream of NMDA receptors. While both RasGRFs are known to contribute to neuronal Ras/Erk signaling *in vivo*, evaluation of Erk activity following NMDA stimulation in acute hippocampal brain slices from knockout animals revealed larger deficiencies in NMDA receptor-mediated Erk activation in $RasGRF2^{-/-}$ mice than in $RasGRF1^{-/-}$ mice (Li and others 2006). Conversely, hippocampal slices from $RasGRF1^{-/-}$ mice show reduced NMDA receptor-mediated activation of the Rac effector p38 MAP kinase, while recordings from $RasGRF2^{-/-}$ slices are indistinguishable from wildtype (Fig.7).

Although basal synaptic transmission appears to be normal in hippocampal slices from mice (P25–36) lacking both RasGRF1 and RasGRF2, expression of both LTP and LTD is blunted in hippocampal slices from adult animals; this deficit is not seen in P14–18 brains (Li and others 2006). Electrophysiological analysis of single knockout brains revealed that hippocampal LTD, but not LTP, is disrupted in $RasGRF1^{-/-}$ mice, while LTP, but not LTD, is impaired in $RasGRF2^{-/-}$ mice (Li and others 2006) (Fig.6B). Further manipulation suggests that these effects are mediated by different NMDA receptor subtypes. APV

blockade of NMDA receptors blocks NMDA-mediated activation of Erk, but not p38, while selective blockade of NR2B-containing NMDA receptors with ifenprodil prevents NMDA-mediated p38 activation with no effect on Erk activation. Thus, electrophysiological and biochemical studies using knockout animals suggest that RasGRF2 signals predominantly through the Ras/Erk pathway in response to NR2A-containing NMDA receptor activation and contributes to LTP induction. Conversely, RasGRF1 contributes mostly to NMDA receptor-dependent LTD via Rac/p38 activation downstream of NR2B-containing NMDA receptor activation (Krapivinsky and others 2003; Li and others 2006) (Fig.7).

Furthermore, cortical Ca^{2+}-permeable AMPA receptor currents are reduced in RasGRF1$^{-/-}$ and 2$^{-/-}$ knockout animals and are abolished in double knockout animals. Both RasGRF proteins co-immunoprecipitate with the GluR1 subunit, but not the GluR2 subunit, of the AMPA receptor in lysates from cortical brain slices following bath application of AMPA (Tian and Feig 2006). Tian et al. also showed that RasGRF expression is required for Ras/Erk signaling following activation of Ca^{2+}-permeable AMPA receptors in the adult brain (Tian and Feig 2006).

Disease relevance

Increased RasGRF1 expression is seen in the striatum, cortex and cerebellum following chronic cocaine, amphetamine or THC administration (Fasano and others 2009; Parelkar and others 2009; Rubino and others 2005; Rubino and others 2006; Zhang and others 2007). Furthermore, RasGRF1$^{-/-}$ mice display decreased locomotor sensitization and conditioned place preference for cocaine (Fasano and others 2009). RasGRF1 may also be involved in CB1 cannabinoid receptor adaptation and drug tolerance following THC administration (Rubino and others 2005; Rubino and others 2006). RasGRF1$^{-/-}$ mice and non-human primates expressing dominant negative forms of RasGRF1 are significantly less susceptible to L-dopa-induced dyskinesias associated with dopamine replacement therapies (Fasano and others 2010), and mice lacking both full-length isoforms have higher susceptibility to cerebral ischemia.

Collybistin

Discovery and expression

Collybistin [*Arhgef9*; human *hPEM2* (Posterior End Mark-2), *ARHGEF9*] was discovered in a yeast two hybrid screen for gephyrin interactors (Kins and others 2000); gephyrin, a scaffolding protein, plays an essential role in the postsynaptic clustering of $GABA_A$ and glycine receptors, both of which are ligand gated inhibitory ion channels. The collybistin gene is located on the X chromosome. In the rat, alternative splicing generates collybistin transcripts that encode functionally distinct proteins (Fig.8A). Some lack the SH3 domain that precedes the catalytic DH/PH domain and three different C-termini (CB1, CB2, CB3) have been identified; $CB2_{SH3+}$ and $CB3_{SH3+}$ are the major splice variants in the rat (Harvey and others 2004). Two promoters generate hPEM2, producing slightly different sequences that precede the SH3 domain (Harvey and others 2004).

Collybistin transcripts are highly expressed in neurons throughout the adult brain and spinal cord, with little expression outside of the nervous system. Collybistin is expressed in all cerebellar neurons, with highest levels in Purkinje cells (Kneussel and others 2001). Expression of collybistin is upregulated during the time of major neuronal differentiation and synaptogenesis and it is thought to play a role in post-mitotic neurons (Kneussel and others 2001; Tolias and others 2011). Collybistin immunostaining is strong in dendrites, with very little in cell body (Tyagarajan and others 2011); Collybistin is detected at only a

subset of gephyrin-positive inhibitory synapses containing all major $GABA_AR$ subunits (Patrizi and others 2011).

Protein structure and signaling

The DH domain of collybistin activates Cdc42, but does not activate Rac1 or RhoA; this domain is involved in binding gephyrin (Kiraly and others 2010a; Tolias and others 2011; Tyagarajan and others 2011). The SH3 domain of collybistin interacts with neuroligin-2, a transmembrane protein that interacts with presynaptic neurexins localized at GABAergic synapses (Chiou and others 2011), and the PH domain binds selectively with the phosphoinositide PI3P (Patrizi and others 2011). Initial comparisons of collybistin variants with and without the SH3 domain suggested an inhibitory role for this region: $CB2_{SH3-}$ forms a ternary complex with gephyrin and Cdc42 while $CB2_{SH3+}$ does not (Tyagarajan and others 2011). Other work argues that all four major isoforms of Collybistin concentrate at GABAergic synapses (Chiou and others 2011).

Collybistin plays a role in localizing gephyrin, which is thought to form a scaffold beneath the plasma membrane that contributes to the anchoring of receptors. Gephyrin, which interacts with microtubules, actin filaments and many additional proteins, plays an essential role in the formation of postsynaptic $GABA_A$ and glycine receptor clusters. The PH domain of collybistin is essential for gephyrin clustering and for interaction with PI3P, but Cdc42 activation by collybistin does not appear to be essential (Tyagarajan and others 2011).

In collybistin$^{-/-}$ mice, a subset of the GABAergic synapses fail to form normally, but glycinergic synapses are unaffected. The effect on $GABA_A$ receptors is both subtype and region-specific: γ2-containing $GABA_A$ receptor levels drop in the basolateral amygdala and hippocampus, but not in the brainstem or cerebellum (Papadopoulos and others 2007). In affected areas, the absence of collybistin results in a decrease in the density of gephyrin clusters at postsynaptic densities and in decreased staining for the γ2 and α2 subunits of the $GABA_A$ receptor. Why the effects are so regionally specific is not yet clear.

Behavior

Given its role in inhibitory receptor clustering, it is not surprising that collybistin$^{-/-}$ mice have significantly fewer $GABA_A$ receptor clusters in the amygdala and hippocampus than wt mice (Papadopoulos and others 2007). Surprisingly, spinal cord and brainstem Gly receptors are properly assembled (Tyagarajan and others 2011). The number of postsynaptic gephyrin puncta was dramatically decreased in the CA1 region of the hippocampus, while the decreases were evident but much less striking in other regions such as the amygdala; paradoxically, the number of VIAAT puncta (presynaptic marker for GABA terminals) was normal (Papadopoulos and others 2007; Jedlicka and others 2009).

Consistent with loss of $GABA_A$ receptor clusters in the amygdala and the hippocampus, Collybistin$^{-/-}$ mice have impaired spatial learning, reduced exploratory behavior, and increased anxiety-like behavior (Papadopoulos and others 2007). Time spent in the open arm of the elevated plus maze was dramatically reduced. The Barnes maze is learned well by wildtype mice, but not by Collybistin$^{-/-}$ mice. Motor performance was normal in Collybistin$^{-/-}$ mice, consistent with normal glycinergic inhibition (Papadopoulos and others 2007).

Electrophysiology and neuronal function

Electrophysiological recordings from collybistin$^{-/-}$ mice have been carried out *in vitro* and *in vivo* (Papadopoulos and others 2007). The frequency and amplitude of GABAergic miniature inhibitory postsynaptic potentials (mIPSPs) in CA1 pyramidal neurons were both

decreased in Collybistin$^{-/-}$ mice. As expected, the field EPSP slope in CA1 is dramatically increased in wildtype mice in the presence of picrotoxin and only a blunted increase is seen in CB$^{-/-}$ mice (consistent with loss of necessary clustering of $GABA_AR$) (Jedlicka and others 2009). LTP induction using theta-burst stimulation of the CA3-CA1 hippocampal collateral pathway produces an enhanced response in Collybistin$^{-/-}$ mice (Fig.8B); the difference between genotypes is eliminated by picrotoxin, again consistent with diminished GABA function in CB$^{-/-}$ mice. A low frequency stimulation paradigm that produces LTD in wildtype mice does not do so in Collybistin$^{-/-}$ mice.

Disease relevance

Harvey et al. (Harvey and others 2004) identified a mutation in the SH3 domain of collybistin when examining 32 hereditary hyperekplexia (excessive startle response) patients; the child with the mutation survived until age 4 but suffered from tonic seizures provoked by tactile stimulation and was severely mentally retarded. Expression of $CB3_{SH3+}$ bearing this mutation results in loss of endogenous gephyrin clusters and mislocalization of $GABA_A$ receptors (Papadopoulos and others 2007). Expression of $CB2_{SH3+}$ mutated at the same site does not support gephyrin localization to the cell surface (Tyagarajan and others 2011). Loss of function mutations in the *ARHGEF9* gene lead to mental retardation and refractory epilepsy in both boys and heterozygous girls (Harvey and others 2004; Shimojima and others 2011).

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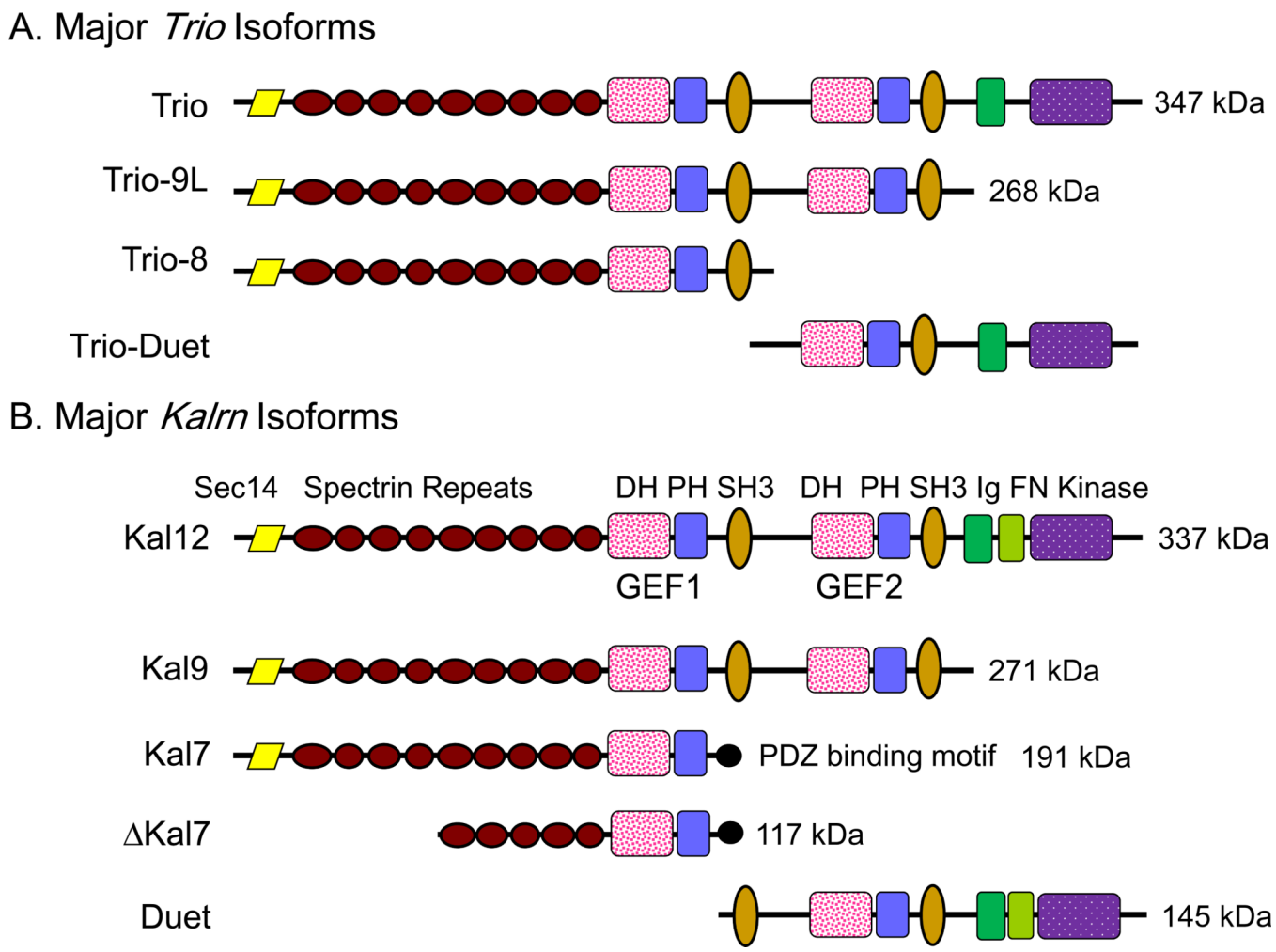

Figure 1. Splice Variants of Kalirin and Trio

Structural features identified in Kalirin and Trio are drawn to scale; the molecular weights of the intact proteins are indicated. Mouse *Kalrn* is on chromosome 16 and human *KALRN* is on chromosome 3. Mouse *Trio* is on chromosome 15 and human *TRIO* on chromosome 5. Only the major adult isoforms of Kalirin and Trio, which arise from alternate splicing, are shown. Some of the isoforms are very tissue-specific; Kal7 is expressed exclusively in neurons and Trio-Duet is primarily a cerebellar product, absent from cerebral cortex (McPherson and others 2005). Abbreviations used throughout all figures are: DH, Dbl-homology; PH, pleckstrin homology; SH3, Src3 homology; Ig, immunoglobulin-like; FN, fibronectin-like; PDZ-BD, PDZ binding motif.

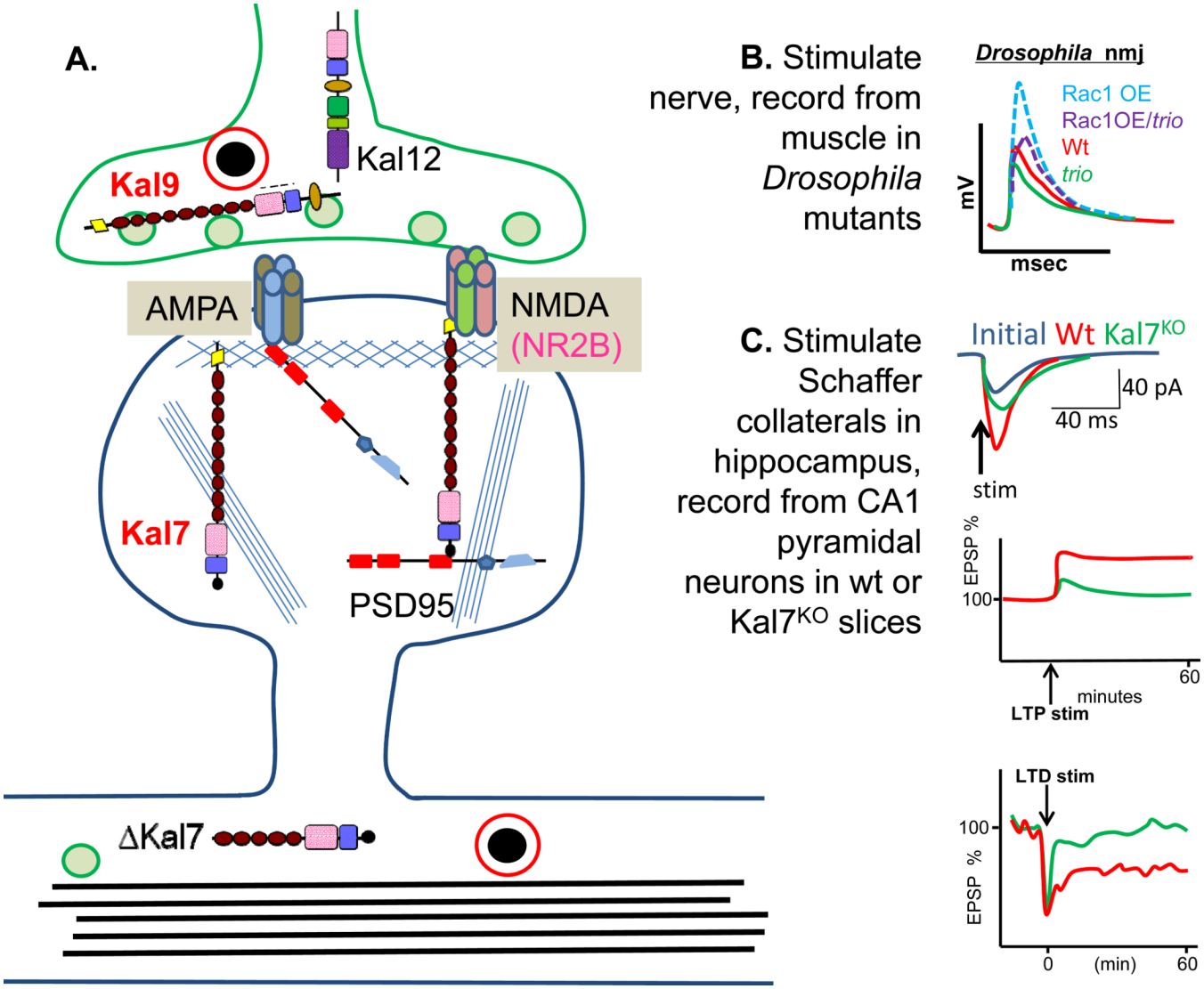

Figure 2. Kalirin and Trio: Synaptic Function

A. The major sites at which different Kalirin isoforms are localized in neurons are indicated. **B.** At the *Drosophila* neuromuscular junction, the dTrio^{KO} (green, *trio*) shows a much smaller endplate potential than the wildtype (red, Wt) (Ball and others 2010). Over-expression of Rac1 in Wt neurons (dashed red, Rac1/Wt) enhances the endplate potential, and over-expression of Rac1 in dTrio^{KO} neurons (dashed green, Rac1/trio) compensates for the absence of *trio*. Thus both Kalirin and Trio are likely to enhance synaptic transmission in part by activating Rac1 via their GEF1 domain. **C.** The Kal7^{KO} mouse shows significantly blunted LTP in hippocampal and cortical neurons compared to wildtype (Wt) mice (green, Kal7^{KO}; red, Wt), and a similar loss of LTP is produced by pharmacological blockade of KalGEF1 (Lemtiri-Chlieh and others 2011; Ma and others 2008b). Kal7^{KO} neurons show little or no LTD.

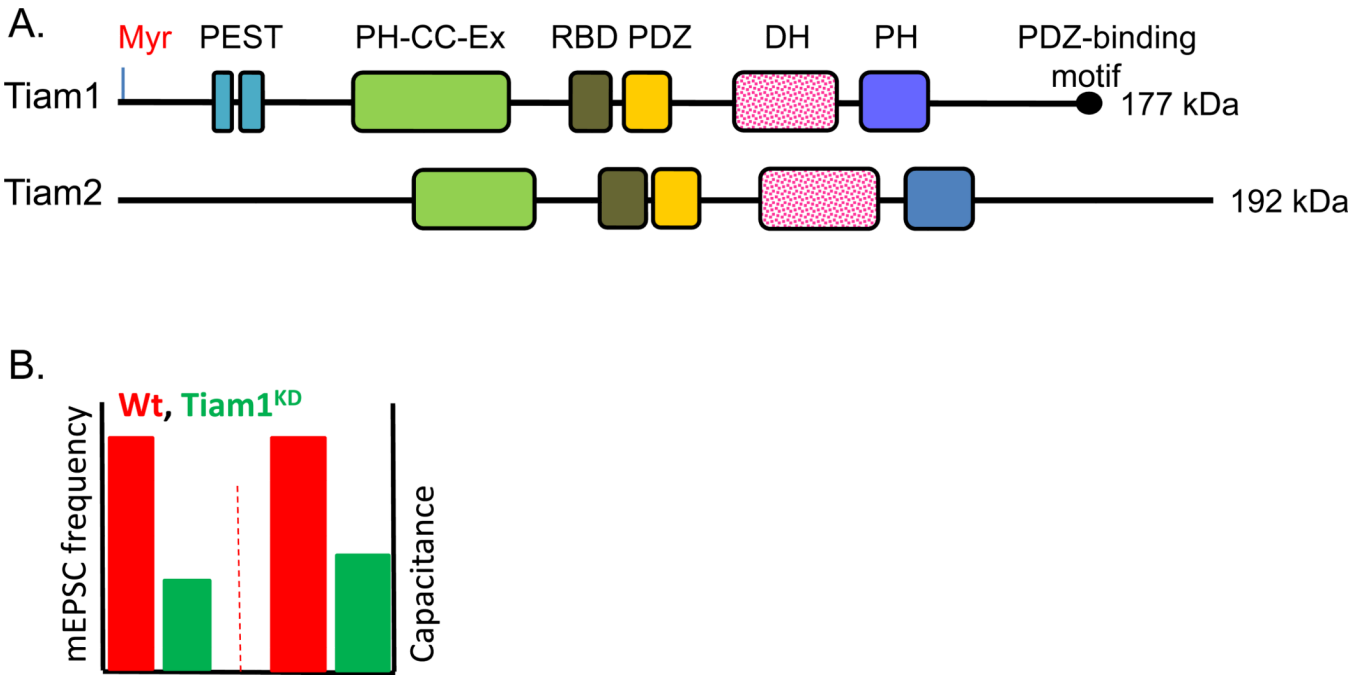

Figure 3. Tiam1 and Tiam2

A. Structure of Tiam1 (*TIAM1*, human 21q22.11) (177 kDa) and Tiam2 (*TIAM2*, human 6q25.2) (192 kDa). **B.** Tiam1 RNAi in rat hippocampal neurons results in a ~62% decrease in mEPSC frequency and a decrease in membrane capacitance compared to the control neurons, suggesting that Tiam1 knockdown results in fewer synapses and smaller cell size (Tolias and others 2005).

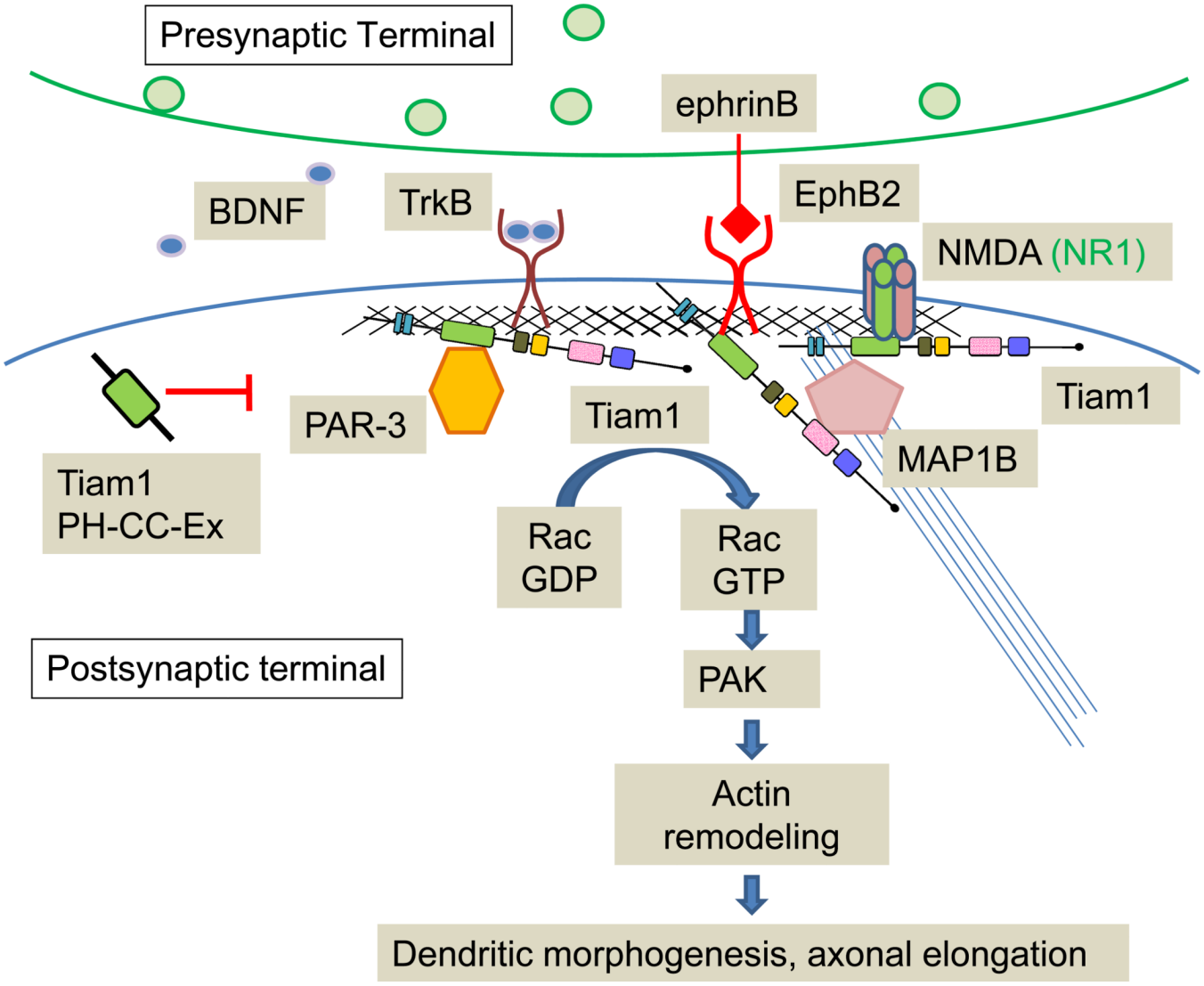

Figure 4. Tiam1: Synaptic Functions

Schematic illustrates proposed signaling mechanisms of Tiam1 at the synapse. Tiam1 interacts with many postsynaptic proteins including the NMDA receptor, EphB, MAP1B, PAR-3 and TrkB, which controls dendritic morphogenesis and axon elongation.

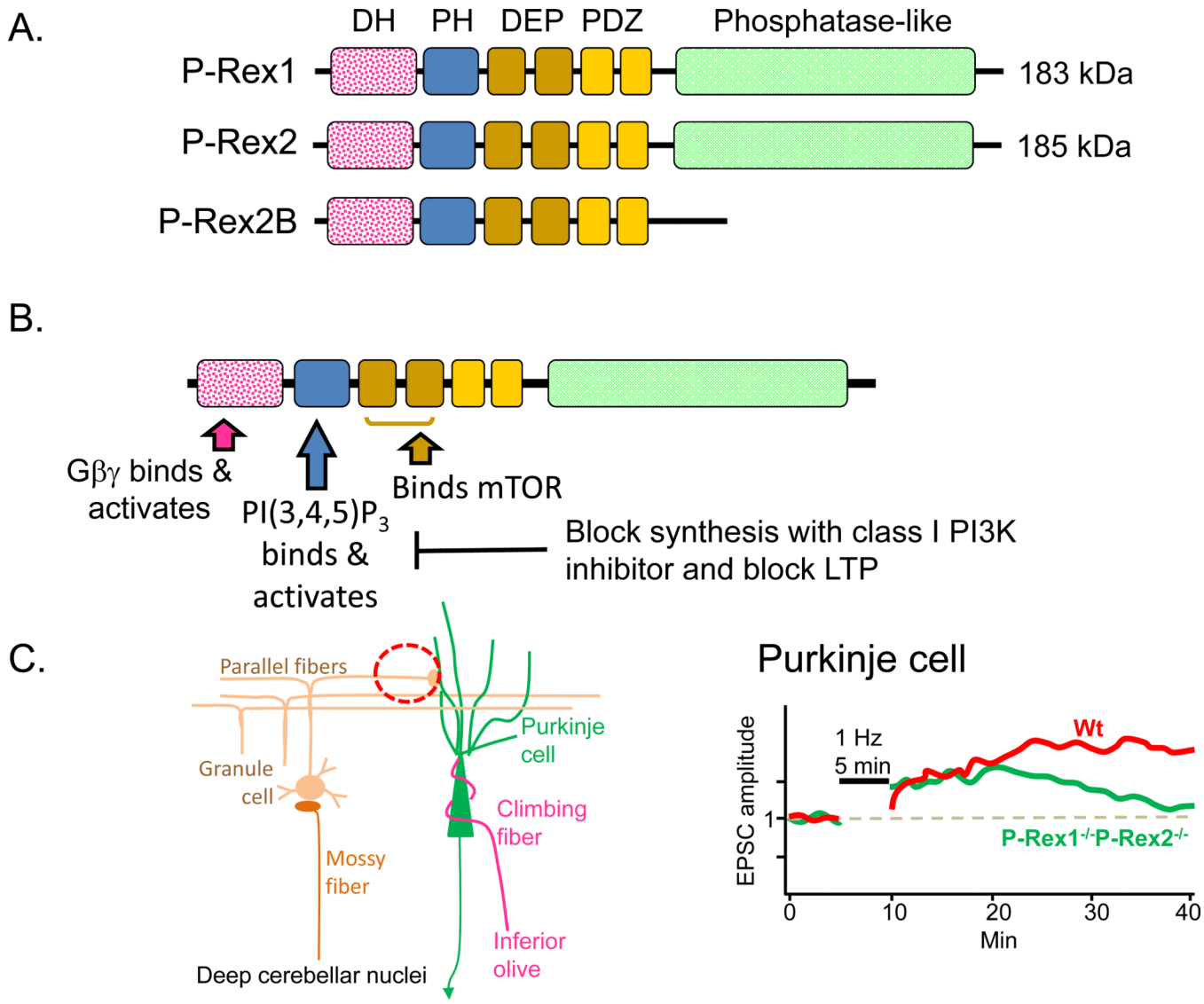

Figure 5. P-Rex1 and P-Rex2

A. Human P-Rex1 (*PREX1*, 20q13.13) (185 kDa) accounts for 0.1% of the cytosolic protein in neutrophils and is also highly expressed in brain (Welch and others 2002). Human P-Rex2 (*PREX2*, 8q13.2) (183 kDa) is not expressed in neutrophils, but is highly expressed in skeletal muscle, heart and placenta. Mouse P-Rex1 and P-Rex2 are on Chr2 and 1, respectively. In addition to a DH and adjacent PH domain, both full-length proteins include two DEP (Disheveled, EGL-10, Pleckstrin) domains, two PDZ domains and an inositol polyphosphate 4-phosphatase-like domain; the phosphatase domain is absent from P-Rex2B, a 980 amino acid splice variant expressed only in the heart (Donald and others 2008). **B.** Key signaling pathways known to affect P-Rex1 function are identified, along with some of its major downstream targets in the nervous system. The DEP and PDZ domains play a role in protein-protein interactions and membrane targeting; phosphorylation of P-Rex1 by PKA disrupts these interactions and diminishes the ability of Gβγ to bind to and activate P-Rex1 (Balamatsias and others 2011). **C.** The cell types involved in the parallel fiber Purkinje cell synapses examined electrophysiologically are illustrated (red dashed circle). Parallel fiber stimulation causes LTP at wildtype synapses; while P-Rex1$^{-/-}$P-Rex2$^{-/-}$ mice produce a

similar response immediately after stimulation, the response is not sustained (Jackson and others 2010).

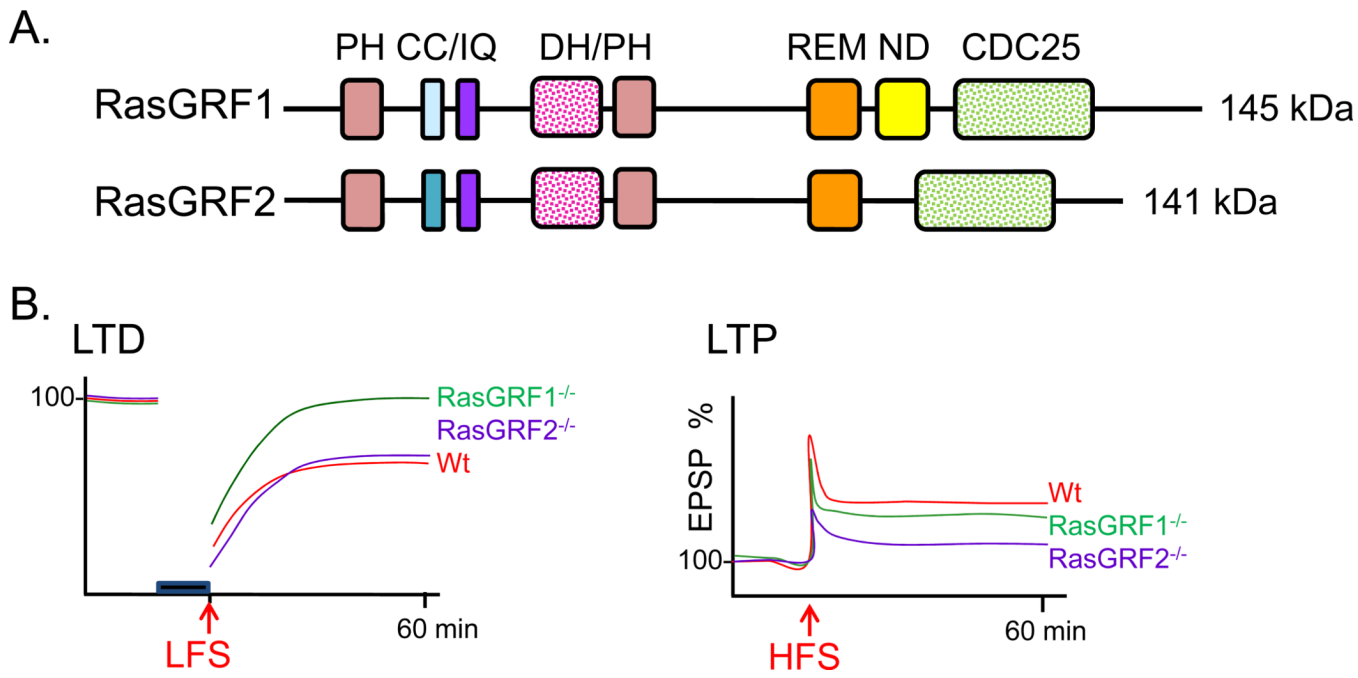

Figure 6. RasGRF1 and RasGRF2

A. RasGRF1 and RasGRF2 are encoded by separate genes (*RASGRF1* and *RASGRF2*), respectively located on chromosomes 15 and 5 in human (Chromosomes 9 and 13 in mouse). Key features of RasGRF1 and RasGRF2 are identified: CC, coiled coil; IQ, Ca^{2+}/calmodulin-binding domain; REM, Ras exchange motif; ND, neuronal domain; CDC25, Ras GEF domain. **B.** Electrophysiological recordings revealed significantly impaired LTP but normal LTD in slices from the hippocampus of P25 and older RasGRF2$^{-/-}$ mice, while LTD but not LTP was disrupted in hippocampal slices from RasGRF1$^{-/-}$ mice (Li and others 2006).

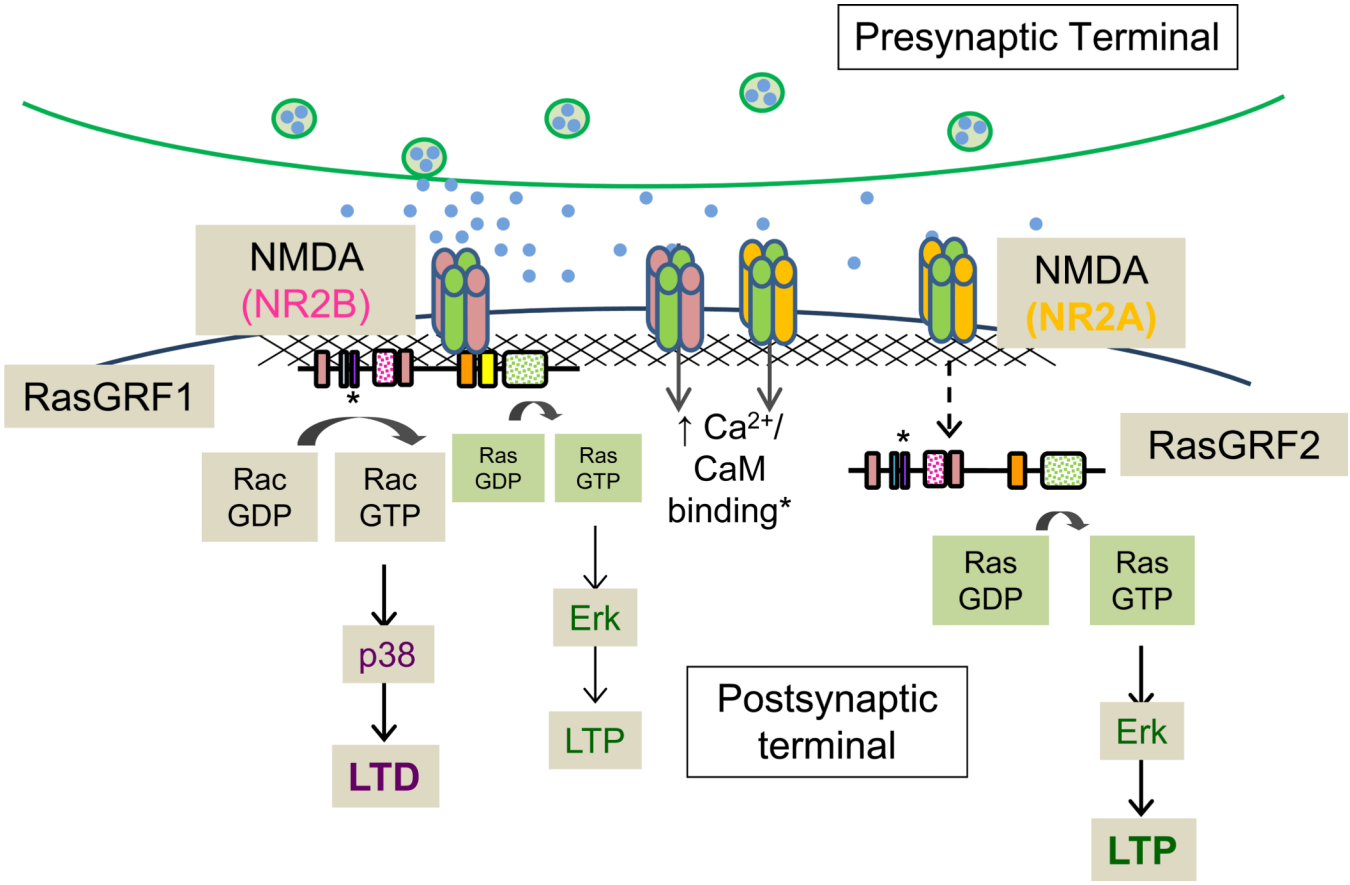

Figure 7. Distinct roles for RasGRF family members in NMDA-mediated long term plasticity

Schematic illustrates proposed signaling mechanisms of the major RasGRF family members, RasGRF1 and RasGRF2. While both proteins are known to contribute to NMDA receptor-mediated Erk activity *in vivo*, data from biochemical and electrophysiological experiments suggest that RasGRF1 acts downstream of NR2B-containing NMDA receptors and signals predominantly through Rac and p38 to promote LTD in hippocampal slices. RasGRF2, however, signals almost exclusively through Ras and Erk following activation of NR2A-containing NMDA receptors to promote LTP.

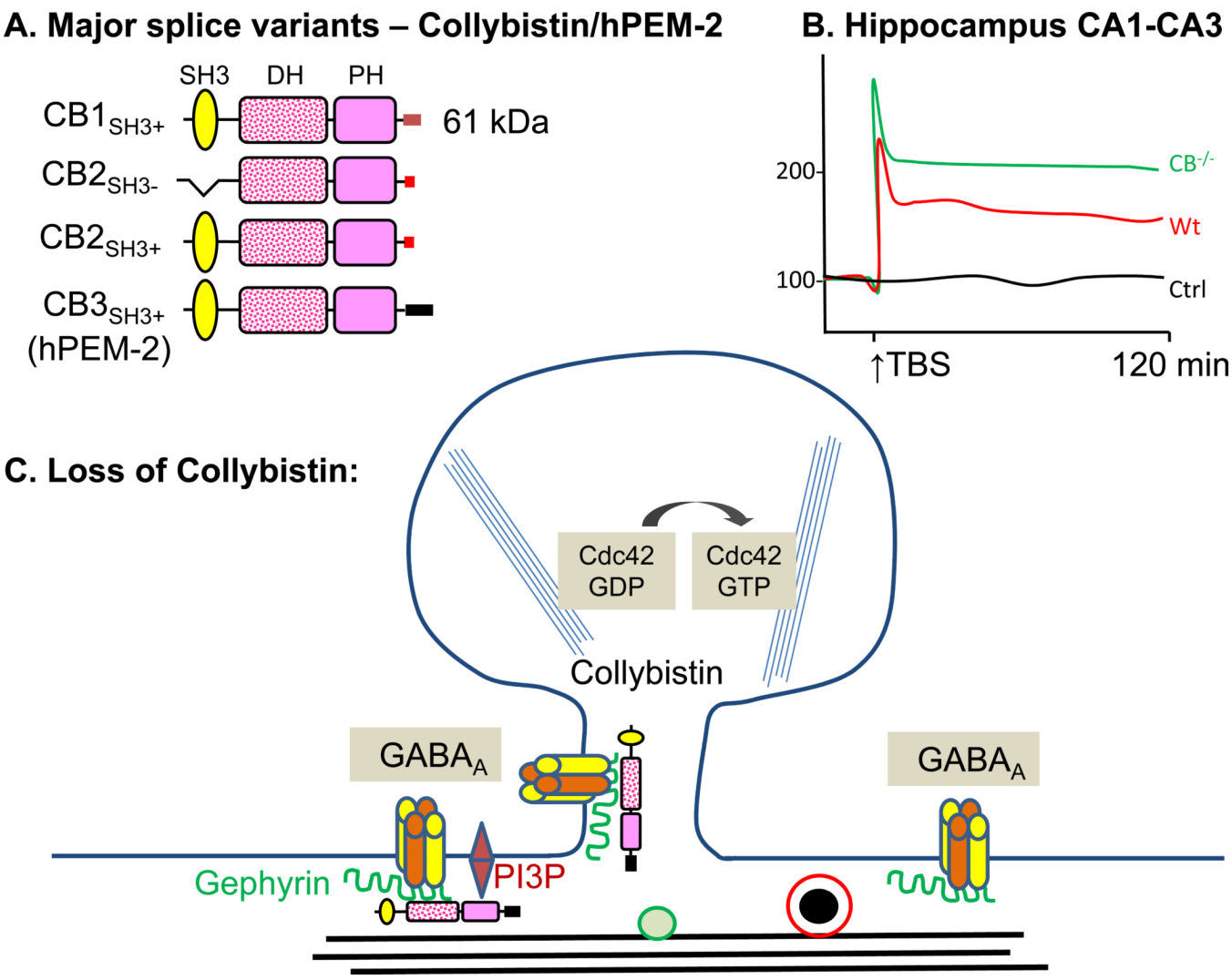

Figure 8. Collybistin. Collybistin structure and synaptic function

A. The major splice variants of rat collybistin (CB; Arhgef9) are shown; the human homologue, hPEM-2 (*ARHGEF9*) has alternate promoters, but does not have similar splice variants (Harvey and others 2004). **B.** Slices prepared from collybistin$^{-/-}$ mice show exaggerated hippocampal LTP; theta-burst stimulation was applied to fibers from CA3 neurons and fEPSPs were recorded in the stratum radiatum of the CA1 region (Papadopoulos and others 2007). The genotypic difference was abrogated in the presence of picrotoxin, a $GABA_A$ receptor blocker, which increased potentiation only in wildtype slices. **C.** Collybistin localizes the scaffolding protein gephyrin, which in turn clusters $GABA_A$ and the group of GlyR receptors (as depicted).