Review

Twenty Years of SynGAP Research: From Synapses to Cognition

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SynGAP is a potent regulator of biochemical signaling in neurons and plays critical roles in neuronal function. It was first identified in 1998, and has since been extensively characterized as a mediator of synaptic plasticity. Because of its involvement in synaptic plasticity, SynGAP has emerged as a critical protein for normal cognitive function. In recent years, mutations in the *SYNGAP1* gene have been shown to cause intellectual disability in humans and have been linked to other neurodevelopmental disorders, such as autism spectrum disorders and schizophrenia. While the structure and biochemical function of SynGAP have been well characterized, a unified understanding of the various roles of SynGAP at the synapse and its contributions to neuronal function remains to be achieved. In this review, we summarize and discuss the current understanding of the multifactorial role of SynGAP in regulating neuronal function gathered over the last two decades.

Introduction

SynGAP was first identified, cloned, and characterized in 1998 by two independent laboratories (Chen et al., 1998; Kim et al., 1998). One study identified SynGAP following a yeast two-hybrid screen for novel PDZ-interacting proteins. The screen specifically identified proteins from a hippocampal cDNA library that interact with the third PDZ domain of SAP102, a member of the membrane-associated guanylate kinase (MAGUK) superfamily of proteins (Kim et al., 1998). This study characterized SynGAP as a synaptically localized GTPase-activating protein (GAP) that could enhance the intrinsic GTPase activity of the signaling enzyme H-Ras, accelerating its inactivation. SynGAP was independently isolated and cloned through purification and mass spectrometry of tryptic peptide sequences from a 130 kDa protein in rat postsynaptic density (PSD) (Chen et al., 1998). Both studies found the expression of SynGAP mRNA and protein to be restricted primarily to the brain (Chen et al., 1998; Kim et al., 1998). Moreover, protein and mRNA levels were higher in forebrain regions than in hindbrain regions in mice (Kim et al., 1998). SynGAP was shown to be a substrate for CaMKII, a key regulator of synaptic plasticity that is critically important for learning and memory (Lisman, 1994; Chen et al., 1998). Finally, both of these studies showed that the C-terminal PDZ-binding motif (PBM) of SynGAP interacts with PSD-95, a major scaffolding protein bound by many PSD proteins (Chen et al., 1998; Kim et al., 1998). This interaction is likely important for the synaptic localization and PSD enrichment of SynGAP (Fig. 1). It would later be discovered that this PBM-containing molecule represents only one of many structural isoforms of SynGAP, which may have unique functions (Fig. 2). Using distinct biochemical methods, these two founding studies of SynGAP identified a curiously synaptically enriched enzyme with the potential to play a role in the regulation of the biochemistry underlying synaptic plasticity.

SynGAP structure and function

SynGAP is an exceedingly abundant constituent of the PSD. Indeed, quantitative proteomic analyses have revealed SynGAP to be one of the most highly abundant proteins in the PSD, reaching copy numbers that are surpassed only by CaMKII α and the PSD-95 family proteins (Sugiyama et al., 2005; Cheng et al., 2006; Sheng and Kim, 2011). Its strikingly high abundance is a clue to its intimate involvement in synaptic function and also is suggestive of unique biochemical and biophysical properties.

Structure

SynGAP protein is encoded by the *SYNGAP1* gene and is expressed as numerous structural isoforms resulting from differential transcriptional start sites and post-transcriptional processing. The first observed variations (one on the N terminus and one on the C terminus) were revealed in experiments demonstrating that endogenous SynGAP protein runs at multiple different molecular weights on SDS-PAGE gels (Chen et al., 1998). The observation of C-terminal structural variants was later confirmed when several variants were cloned from a rat cDNA library (Li et al., 2001). A comparison of the new mRNA sequences to sequences identified previously suggested alternative splicing as a mechanism for generating structural variants (Li et al., 2001). The observation of N-terminal SynGAP structural variants was confirmed and expanded using a combination of mouse and rat cDNA library screens, 5' rapid amplification of cDNA ends (5'-RACE) of RNA isolated from mouse forebrain, and mass spectrometry of mouse brain samples (Li et al., 2001; McMahon et al., 2012). Three N-terminal isoforms (A–C) result from alternative transcriptional start site usage (Fig. 2B), and at least four C-terminal SynGAP splice variants ($\alpha 1$, $\alpha 2$, β , and γ) are currently known (Fig. 2C). SynGAP isoforms are often named using a combinatorial designation referring to their N- and C-terminal identities (e.g., SynGAP A $\alpha 1$). The $\alpha 1$ isoform is the only isoform that contains the C-terminal PBM, which allows SynGAP to bind PDZ-domain-containing MAGUK family proteins in the PSD. The numerous SynGAP isoforms display distinct distribution patterns in neuronal subcellular compartments, and have been observed to differentially regulate synaptic strength in cultured neurons (Li et al., 2001; McMahon et al., 2012). The mechanisms underlying these differences are not yet completely understood.

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Figure 1. SynGAP localization in cultured neurons. **A**, Overexpression of GFP-SynGAP $\alpha 1$ (green) and mCherry (magenta) in a DIV 18 cultured rat hippocampal neuron. Inset, Robust enrichment of GFP-SynGAP $\alpha 1$ in dendritic spines. **B**, Distribution of SynGAP in various subcellular fractions from DIV 19 cultured rat cortical neurons. Subcellular fractionation was performed as described by Diering et al. (2014). SynGAP $\alpha 1$ is enriched in the PSD fraction and is found in much lower abundance in the cytosolic fraction (S2). Immunoblots are shown for SynGAP $\alpha 1$ and PSD-95, a PSD marker and binding partner of SynGAP. S1, Postnuclear supernatant fraction; P2, crude membrane fraction.

SynGAP gene and protein structure are also discussed in detail in two recent reviews about SynGAP (Jeyabalan and Clement, 2016; Kilinc et al., 2018).

Numerous reports have suggested that non- $\alpha 1$ isoforms of SynGAP can attain similar degrees of synaptic enrichment to that of SynGAP $\alpha 1$ despite the lack of a C-terminal PBM (Li et al., 2001; Yang et al., 2013). However, it is unclear how these isoforms associate with the PSD. One possible mechanism involves the potential multimerization of SynGAP isoforms via coiled-coil domain interactions (Zeng et al., 2016) (Fig. 2). Thus, SynGAP $\alpha 1$ -containing trimers may recruit other C-terminal SynGAP isoforms to the PSD, although it is currently unknown whether SynGAP trimers can contain more than one SynGAP species. Another possible mechanism involves the association of SynGAP isoforms with synaptic molecules other than PSD-95. For example, SynGAP β , which does not interact with PSD-95, has been reported to interact with the CaMKII α -subunit in its inactive, nonautophosphorylated form (Li et al., 2001). In addition, all SynGAP isoforms contain a proline-rich SH3-binding domain that potentially allows SynGAP to associate with many SH3-domain-containing PSD proteins. However, little is known about functional interactions between this region and other synaptic proteins. Interestingly, the region of SynGAP that is C-terminal to its GAP domain is largely structurally disordered (Fig. 2D). Structural disorder has been shown to impart unique structural and biochemical properties to proteins, including but not limited to context-dependent regulation of protein–protein interaction profiles, regulation of alternative splicing, and the ability to assemble dynamic protein complexes by liquid–liquid phase separation (LLPS) (Wright and Dyson, 2015; Turoverov et al., 2019).

However, the role of intrinsic disorder within SynGAP remains to be thoroughly explored.

Expression and localization

SynGAP protein is primarily expressed in the brain, although SynGAP protein can be detected at low levels in other tissues, including lung, kidney, and testes (Chen et al., 1998). Within the brain, expression is highest in forebrain structures, including the cortex, hippocampus, and olfactory bulb (Kim et al., 1998; Porter et al., 2005). Expression of SynGAP mRNA and protein peaks at times of robust synaptogenesis (Porter et al., 2005; McMahon et al., 2012). The SynGAP $\alpha 1$ isoform exhibits a particularly high degree of synaptic enrichment, presumably due to its C-terminal PBM (Fig. 1) (Chen et al., 1998; Nonaka et al., 2006). Electron microscopy has revealed that, under unstimulated baseline conditions, SynGAP localizes primarily to the core region of the PSD within 40 nm of the postsynaptic plasma membrane (Sheng and Kim, 2011; Yang et al., 2011). The various SynGAP isoforms exhibit distinct expression profiles that are brain-region- and cell-type-specific. For example, while SynGAP $\alpha 1$ and β isoforms are primarily localized to synapses in forebrain neurons, the $\alpha 1$ isoform is localized almost exclusively to excitatory synapses, whereas the β isoform can be observed at both excitatory and inhibitory synapses (Moon et al., 2008). Differences in the spatiotemporal expression profiles of the SynGAP structural isoforms may be clues to their primary functional roles in regulating synaptic plasticity.

Biochemical function

SynGAP was first identified as a Ras-specific GAP (RasGAP) due to sequence similarity between its GAP domain and the GAP domains of other known RasGAPs (Kim et al., 1998). It is a member of a small structurally defined subfamily of RasGAPs that harbor a pleckstrin homology domain and C2 domain upstream of the GAP domain (King et al., 2013). Ras is a superfamily of small GTPases, the members of which are constituents of cellular signaling pathways that regulate a variety of cellular processes, especially those involving growth and survival (Tidyman and Rauen, 2016; Mo et al., 2018; Scheffzek and Shivalingaiah, 2019). Ras proteins are expressed in a variety of tissue types, including neurons, and dysregulation of Ras function has been linked to a plethora of human diseases, including cancers and cognitive disorders (Tidyman and Rauen, 2016; Simanshu et al., 2017; Mo et al., 2018; Kim and Baek, 2019). Biochemically active Ras is bound to GTP, and Ras activity is terminated following hydrolysis of the bound GTP to GDP via intrinsic GTPase activity, which can be accelerated by GAPs (Cherfils and Zeghouf, 2013; Simanshu et al., 2017).

The Ras GAP activity of SynGAP was initially confirmed using *in vitro* GAP activity assays (Chen et al., 1998; Kim et al., 1998). However, the GAP activity of SynGAP has recently been shown to be more complex than previously thought. SynGAP potentiates the GTPase activity of Rap, another small GTPase, and does so more robustly than for Ras (Krapivinsky et al., 2004). The C2 domain immediately upstream of the GAP domain within SynGAP (Fig. 2) is required for this Rap GAP activity (Pena et al., 2008). As such, SynGAP can be considered a multifunctional GAP, enhancing the GTPase activity of a number of small GTPases.

Dual regulation of both Ras and Rap small GTPases has important implications for neuronal function. While Rap and Ras are members of the same small GTPase superfamily, their roles in neuronal physiology appear to oppose one another (Zhu et al., 2002).

Ras is activated following NMDAR-dependent activation of CaMKII in a signaling pathway that promotes the insertion of AMPAR subunits into the post-synaptic plasma membrane (Zhu et al., 2002). Rap, on the other hand, is activated in response to lower levels of Ca^{2+} influx, and contributes to the removal of GluA2-containing AMPARs from the synapse (Zhu et al., 2002). Thus, the functional role of SynGAP at synapses is complex and may depend on its relative regulation of Ras and Rap activity.

Several studies have demonstrated that the GAP activity of SynGAP can be differentially altered by post-translational modifications. SynGAP can be phosphorylated by multiple synaptic protein kinases at ≥ 20 distinct sites, primarily within its C-terminal disordered domain (Walkup et al., 2016). It is phosphorylated by CaMKII at multiple sites, and this phosphorylation enhances its enzymatic activity (Chen et al., 1998; Oh et al., 2004; Dosemeci and Jaffe, 2010). Phosphorylation of SynGAP by CaMKII enhances its Rap1 GAP activity significantly more than its Ras GAP activity, whereas the opposite is true following phosphorylation by CDK5 (Walkup et al., 2015). Polo-like kinase 2 (Plk2), an enzyme known to be involved in the activity-dependent homeostatic scaling of synaptic strength (Seeburg et al., 2008), was also shown to phosphorylate SynGAP directly at multiple sites, to enhance GAP activity toward H-Ras more than Rap1, and to act in concert with CDK5 to enhance H-Ras inactivation more than Rap1 inactivation (Walkup et al., 2018). These data suggest that SynGAP operates within a complex network of signaling cascades, and that SynGAP localization, activity, and function can be tuned by multiple signaling factors to regulate surface AMPAR expression and synaptic plasticity. In this way, SynGAP might be considered a molecular hub for the regulation of synaptic strength at baseline and following neuronal activity.

Importantly, there are nonsynaptic functions of SynGAP that contribute to neuronal development and function. SynGAP has been shown to regulate axon outgrowth in cerebellar granule cells through the regulation of Rab5, another small GTPase (Tomoda et al., 2004). Additionally, SynGAP loss of function has been shown to alter the growth trajectory of developing neurons in mouse cortex (Aceti et al., 2015). These data imply a role for SynGAP in neuronal maturation that is distinct from its roles at the synapse.

SynGAP in synaptic plasticity

Signaling roles

The localization patterns and biochemical functions of SynGAP were very early clues to the intimate involvement of SynGAP in synaptic plasticity. Synaptic strengthening can occur in an activity-dependent manner by a process known as LTP, during which the dendritic spines of stimulated synapses enlarge and

Figure 2. SynGAP structural isoforms. **A**, Schematic diagram of SynGAP protein structure. The core region comprises the C2, GAP, and proline-rich domains (gray). The extreme N- and C-termini contain variable domains whose structure depends on transcriptional and post-transcriptional processing. The remaining pleckstrin homology (PH) domain and coiled-coil (CC) domain (both magenta) are altered in several isoforms. **B**, Schematic diagram of the N-terminal isoforms of SynGAP arising from the use of alternative transcriptional start sites. The PH domain is partially truncated in the C isoform. **C**, Schematic diagram of the C-terminal isoforms of SynGAP arising from alternative splicing. The CC domain is partially truncated in the β isoform. **D**, Intrinsic disorder probability plotted as a function of amino acid position along the full length of the SynGAP protein sequence starting with the beginning of the A isoform. Disorder probabilities for all four C-terminal isoforms, $\alpha 1$ (red), $\alpha 2$ (orange), β (blue), and γ (purple), are shown. Disorder probabilities were calculated using IUPred2A (Mészáros et al., 2018).

accumulate postsynaptic AMPARs (Huganir and Nicoll, 2013; Diering and Huganir, 2018). The localization of SynGAP to the PSD also changes in an activity-dependent manner (Yang et al., 2011; Araki et al., 2015). Early studies of activity-dependent SynGAP dynamics were performed on cultured neurons using immunogold labeling with a pan-SynGAP antibody followed by electron microscopy (Yang et al., 2011). Following potassium chloride-induced depolarization, SynGAP translocates from the core region of the PSD to the contiguous network and adjacent cytoplasmic compartment outside of the PSD complex (Yang et al., 2011).

These findings were confirmed and expanded upon in experiments using confocal imaging of SynGAP dynamics in living cultured neurons. Following chemically induced LTP (chem-LTP), SynGAP is phosphorylated by CaMKII in an NMDAR-dependent manner, and this decreases the affinity of SynGAP for PSD-95 and results in the rapid dispersion of SynGAP away from the PSD (Fig. 3A,B) (Araki et al., 2015). This dispersion represents the release of the brake on Ras signaling activity near the PSD allowing the emergence of LTP-associated signaling and structural changes to the synapse, including but not limited to activation of ERK for downstream AMPAR insertion (Zhu et

Figure 3. SynGAP dynamics. **A**, Representative images of a segment of a dendrite of a cultured rat hippocampal neuron expressing GFP-SynGAP and mCherry at basal state (left column) and following chemLTP stimulation (right column). The GFP-SynGAP signal is rapidly reduced in a dendritic spine following chemLTP (yellow arrow). The volume of the same dendritic spine is increased following chemLTP, as shown by the mCherry signal. **B**, Quantification of the GFP-SynGAP and mCherry dynamics in **A**. These data are reprinted from Araki et al. (2015) with permission. **C**, Expression of Azurite-tagged SynGAP C terminus (Az-SynGAP CC-PBM WT) (blue) and full-length PSD-95-mCherry (red) in a HEK 293T cell. Coexpression results in the formation of spherical cytoplasmic condensates containing both proteins. Image brightness and contrast were adjusted to show the presence of the diffuse soluble phase of both proteins outside of granules. **D**, Enlarged channel-split images of the boxed cytoplasmic condensate in **C** before and after photobleaching with a 561 nm laser. The PSD-95-mCherry signal rapidly recovers fluorescence following photobleaching, indicating rapid exchange of molecular constituents with the surrounding cytoplasm, a property common to liquid-like biomolecular condensates. These data are a replication of data from Zeng et al. (2016). **E**, Quantification of the normalized mean fluorescence intensity following FRAP experiments plotted as the mean $\pm$ SEM ($N = 2$ granules in 2 cells).

al., 2002; Rumbaugh et al., 2006; Wang et al., 2013) and activation of the Rho GTPase Rac, which promotes actin polymerization and subsequent spine enlargement (Carlisle et al., 2008). Indeed, the magnitude of chemLTP-dependent SynGAP dispersion is correlated with increases in both dendritic spine volume and synaptic surface AMPAR number (Fig. 3A,B) (Araki et al., 2015). Importantly, SynGAP dispersion and its downstream effects are blocked by mutating the CaMKII phosphorylation sites of SynGAP (Araki et al., 2015). These results demonstrate that dispersion of SynGAP from synapses is a simple model to explain the regulation of synaptic signaling by SynGAP: the physical removal of SynGAP from the PSD upregulates Ras signaling required for LTP expression. This model is supported by experiments using overexpression of the PBM-containing SynGAP $\alpha 1$ isoform in cultured neurons. However, it remains unclear whether other SynGAP isoforms share these fast activity-dependent dynamics and whether the non- $\alpha 1$ isoforms contribute significantly to plasticity-related changes in synaptic and neuronal function. There is some evidence that SynGAP $\alpha 2$ attains comparable PSD enrichment to SynGAP $\alpha 1$, and undergoes activity- and CaMKII-dependent dispersion (Yang et al., 2013). Whether this effect is independent of SynGAP $\alpha 1$ dispersion is not clear.

Potential structural roles

In addition to its enzymatic activity and participation in biochemical signaling pathways that are involved in synaptic plasticity, some evidence for a structural role for SynGAP during plasticity has emerged over the last decade. During plasticity, the PSD undergoes rapid and dramatic structural rearrangement (Meyer et al., 2014; Dosemeci et al., 2016; Lautz et al., 2018; Borczyk et al., 2019). The PSD thickens in response to robust depolarization of cultured neurons and neurons in brain slices, and does so on exquisitely short timescales (Dosemeci et al., 2001; Meyer et al., 2014). The rapid removal of abundant proteins, including SynGAP, from the PSD implies a fast molecular rearrangement driven at least in part by mass action. It is possible that the removal of a large number of binding constituents of the PSD could promote the association of other molecules with the newly available binding domains. The idea that binding “slots” could be freed up and dynamically reoccupied at synapses has been a leading model for synaptic plasticity for over a decade (Shi et al., 2001; Lisman and Raghavachari, 2006; Kessels et al., 2009; Makino and Malinow, 2009; Hugarir and Nicoll, 2013; Diering and Hugarir, 2018). It is thought that MAGUK family proteins, such as PSD-95, represent the central physical platforms on which synaptic molecules can occupy a finite number of binding “slots,” commonly PDZ domains. The synaptic molecules that could dynamically occupy these slots include but are not limited to glutamate receptor subunits, transmembrane proteins such as transmembrane AMPAR regulating proteins (TARPs), enzymes such as SynGAP, and other PDZ-binding scaffolding molecules. The PSD-95 slot hypothesis is reviewed thoroughly by Opazo et al. (2012).

Because of its abundance, SynGAP may occupy synaptic MAGUK “slots” at baseline, limiting the number of synaptic surface AMPARs, and thus the strength of the synapse. PSDs from brain tissue samples of SynGAP haploinsufficient mice exhibit differences in composition, most notably in that they exhibit elevated levels of other PDZ-binding proteins, including TARPs, LRRTM2, and Neuroligin-2 (Walkup et al., 2016). More experiments are required to determine whether these effects are purely structural in nature, or rather result from persistent elevated activity of small GTPases that are normally inhibited by SynGAP.

Other studies have provided hints to a role for SynGAP in the regulation of PSD structure from a biophysical perspective. These studies showed that SynGAP and PSD-95 undergo LLPS together *in vitro* and in live cells, in turn forming a dynamic macromolecular complex that is spatially distinct from the surrounding cytoplasm (Fig. 3C–E) (Zeng et al., 2016, 2019a). Biological LLPS is a phenomenon in which biological macromolecules, such as proteins and RNA, condense into self-contained higher-order structures with physical properties that resemble Newtonian fluids

(Brangwynne et al., 2009; Shin and Brangwynne, 2017). They are sometimes termed “membraneless organelles” because they can contribute to cellular processes in a spatially restricted manner, but without enclosure by a lipid membrane, which is the case for traditional cellular organelles (Shin and Brangwynne, 2017). Due to their lack of membrane enclosure, liquid-like biomolecular condensates can sense and respond to changes in the adjacent environment quickly and dynamically while remaining spatially compartmentalized (Alberti et al., 2019). Because synaptic plasticity is a process that requires postsynaptic structures to be exquisitely sensitive to and responsive to stimulation, LLPS is an attractive mechanism for PSD formation, maintenance, and modulation. However, the link between SynGAP/PSD-95 LLPS and AMPAR dynamics during plasticity remains incompletely understood. More recent work has suggested that SynGAP/PSD-95 LLPS itself might not be required for PSD formation, and that SynGAP is perhaps only one of many molecules in the PSD that undergo multivalency-driven LLPS with PSD scaffolding molecules (Zeng et al., 2018, 2019b).

Requirement of SynGAP for normal synaptic plasticity

Homozygous deletion of SynGAP in mice results in perinatal lethality (Komiyama et al., 2002; Kim et al., 2003). However, SynGAP heterozygosity affords normal survival, and neurons from the embryos of SynGAP homozygous KO mice can be maintained in cell culture for several weeks (Kim et al., 2003; Vazquez et al., 2004). Hippocampal brain slices from adult SynGAP haploinsufficient mice exhibit deficits in LTP induced by theta burst stimulation (Kim et al., 2003; Ozkan et al., 2014) and spike pairing (Komiyama et al., 2002), whereas LTD and basal synaptic transmission properties are normal (Komiyama et al., 2002; Kim et al., 2003). Interestingly, restoration of SynGAP expression in adult SynGAP heterozygous mice can rescue this LTP deficit, directly implicating SynGAP in the regulation of LTP (Ozkan et al., 2014). Neuronal cultures from SynGAP homozygous KO mouse embryos exhibit a decrease in the number of AMPAR-lacking “silent synapses,” and larger dendritic spines that develop precociously compared with those in cultured WT mouse neurons (Kim et al., 2003; Vazquez et al., 2004). These findings are consistent with data from experiments testing the effect of SynGAP knockdown on dendritic spine size and AMPAR content in cultured neurons, where the amount of SynGAP in spines is inversely correlated with spine size and synaptic surface AMPAR number (Araki et al., 2015). SynGAP knockdown neurons undergo derepression of Ras signaling and display enhanced ERK activity (Rumbaugh et al., 2006), which is required for activity-dependent AMPAR insertion (Zhu et al., 2002). These experiments have implications for plasticity deficits in the context of disease-associated *SYNGAP1* haploinsufficiency, where aberrant Ras signaling and PSD structural differences could potentially underlie deficits in cognition.

SynGAP in health and disease

Over the last 10 years, SynGAP has been increasingly implicated as a risk gene for neurodevelopmental disorders, such as intellectual disability (ID), autism spectrum disorders (ASDs), schizophrenia, and other disorders affecting cognitive function (Jeyabalan and Clement, 2016). *De novo* mutations in the *SYNGAP1* gene were first associated with ID in humans in 2009 (Hamdan et al., 2009). Since then, >200 patients suffering from loss-of-function mutations in *SYNGAP1* have been identified through genetic sequencing (Weldon et al., 2018). Many of these mutations are nonsense or frameshift mutations that cause nonsense-mediated decay of *SYNGAP1* mRNA

or dysfunctional truncated protein products, and likely result in haploinsufficiency. It is estimated that these *SYNGAP1* mutations may account for up to 1% of all cases of nonsyndromic ID (Hamdan et al., 2009). However, with an increase in the number of identified patients harboring *SYNGAP1* mutations, it is now apparent that *SYNGAP1* loss-of-function mutations precipitate a constellation of symptoms that can be classified as a single disorder, Mental retardation, autosomal dominant 5 (MRD5) (Parker et al., 2015; Agarwal et al., 2019; Holder et al., 2019). ID-causing *SYNGAP1* mutations have been identified along the entire length of the *SYNGAP1* gene, with most of the mutations occurring from exons 3–17 (Vlaskamp et al., 2019) (Fig. 4). Human patients with *de novo* loss-of-function *SYNGAP1* mutations suffer from substantial developmental delay, resulting in severe cognitive deficits. Affected children often do not walk unaided until 3 years of age and exhibit hyperexcitable behavior, aggressiveness, abnormal sleep patterns, and repetitive behaviors (Hamdan et al., 2009; Parker et al., 2015; Mignot et al., 2016). Most *SYNGAP1* patients suffer from epileptic seizures, including absence seizures, myoclonic seizures, reflex seizures, and drop attacks (Berryer et al., 2013; Parker et al., 2015; Mignot et al., 2016; Vlaskamp et al., 2019). Many patients also have distinctive myopathic facial features and gastrointestinal symptoms (Hamdan et al., 2009; Vlaskamp et al., 2019).

Physiology and behavior of animal models of SYNGAP1 haploinsufficiency

SynGAP loss-of-function phenotypes appear to be conserved across many vertebrate species, making animal models of SynGAP haploinsufficiency useful not only for probing the roles of SynGAP in physiology and cognition, but also for understanding the roles of SynGAP in disease contexts (Kilinc et al., 2018). Heterozygous SynGAP mice exhibit severe memory deficits, as seen in their performance in the radial arm maze, Morris water maze, and the elevated T-maze tasks (Komiyama et al., 2002; Guo et al., 2009; Muhia et al., 2010; Clement et al., 2012). Conditionally removing SynGAP selectively from excitatory forebrain neurons has been shown to be sufficient to cause behavioral deficits in mice, suggesting that *SYNGAP1*-related disorders result from disruption of the development and function of glutamatergic forebrain neurons (Ozkan et al., 2014). This could potentially have secondary developmental consequences that propel the brain into a pathological state at the circuit level (Ozkan et al., 2014). SynGAP heterozygous mice display dysregulated development of brain sensory systems, from the level of the thalamus through primary somatosensory cortex (Barnett et al., 2006). The developmental dysregulation of cortical circuits appears to arise from aberrant neonatal synaptogenesis, including precocious and accelerated spine morphogenesis, leading to reduced critical period plasticity and altered long-range circuit connectivity (Aceti et al., 2015). This role of SynGAP in dendritic spine development has been corroborated in non-SynGAP animal models of disease. The expression of SynGAP $\alpha 2$ is regulated through stabilization of its mRNA by FUS (Yokoi et al., 2017), a protein best known for its association with the neurodegenerative diseases amyotrophic lateral sclerosis and frontotemporal lobar degeneration (Gao et al., 2017; Zhao et al., 2018). Conditional FUS KO mice exhibit decreased SynGAP $\alpha 2$ protein levels, and exogenous reintroduction of SynGAP $\alpha 2$ in this model rescues behavioral and spine maturation deficits, supporting a role for a single isoform of SynGAP in synaptic development (Yokoi et al., 2017). These data imply a role for SynGAP in brain development, where SynGAP hypofunction may lead to broad dysregulation of brain

Figure 4. ID-associated *SYNGAP1* mutations. *SYNGAP1* mutations associated with ID as collated in Vlaskamp et al. (2019). Mutations are binned and listed according to their respective exon or intron. Exonic mutations are named for the affected amino acid residue (first letter), position (number), and the resultant change to the residue due to the mutation (following letter). Intronic mutations are named for the resultant nucleotide change relative to the *SYNGAP1* reference sequence. *Change resulting in a stop codon. fs*, Frameshift that results in a stop codon downstream of the frameshift site. *SynGAP* protein domains are as they appear in Figure 2. Truncating mutations are listed above the *SYNGAP1* gene and protein structure, and nontruncating missense mutations are listed below. Importantly, the mutations are not evenly distributed across the *SYNGAP1* gene, with most identified pathogenic mutations occurring after the first two and before the final two exons.

development in addition to aberrant synaptic plasticity. However, *SynGAP* hypofunction may also lead to nondevelopmental insults on brain function, as restoration of *SynGAP* function in adult *SynGAP* heterozygous mice has been shown to rescue many *SYNGAP1*-haploinsufficiency-related behavioral and physiological deficits (Creson et al., 2019). The role of *SynGAP* in the etiology of ID and ASDs is thus likely multidimensional. Together, these studies strongly implicate *SynGAP* in synaptic and neuronal development and plasticity, and establish animal models of *SynGAP* loss of function as useful tools for understanding the mechanistic underpinnings of *SYNGAP1*-related disorders.

Numerous reports describe *SYNGAP1* patients as having unique tactile symptoms, including pain hyposensitivity and an unusual affinity for the sensation of water on their skin (Michaelson et al., 2018; Vlaskamp et al., 2019). Sensory deficits, including those that are tactile in nature, are very common features of ASDs and ID (Orefice et al., 2016; Robertson and Baron-Cohen, 2017). *SYNGAP1*-related tactile phenotypes have been captured by a mouse model of *SYNGAP1* haploinsufficiency (Michaelson et al., 2018). In this study, *SYNGAP1* heterozygous mice exhibited sensory processing deficits in somatosensory cortex. These mice show a decrease in sensory-evoked activity in the somatosensory cortex, which was unexpected given that *SYNGAP1* haploinsufficiency has been associated with aberrant enhancement of the strength of individual synapses on the molecular level, and general circuit hyperexcitability on the systems level (Michaelson et al., 2018). These results suggest a CNS-centric mechanism for sensory abnormalities resulting from *SynGAP* loss of function. Interestingly, *SynGAP* has also been observed to be expressed in primary afferent sensory neurons in the peripheral nervous system (Duarte et al., 2011). *SynGAP* haploinsufficient mice display peripheral sensitization in response to capsaicin and, in turn, exhibit capsaicin-induced thermal hyperalgesia at a lower capsaicin dose compared with WT littermates (Duarte et al., 2011). The details surrounding the interplay between central (Michael-

son et al., 2018) and peripheral (Duarte et al., 2011) *SynGAP* dysfunction in the manifestation of sensory processing deficits are only beginning to be elucidated.

Autism

While all patients with *SYNGAP1* loss-of-function mutations exhibit some form of ID, ~50% are codiagnosed with ASD (Berryer et al., 2013; Parker et al., 2015; Mignot et al., 2016; Agarwal et al., 2019; Holder et al., 2019), which has long been linked to synaptic pathophysiology and is often caused by mutations in genes encoding synaptic proteins (Hamdan et al., 2011; Penzes et al., 2011; Berryer et al., 2013; O’Roak et al., 2014; Kilinc et al., 2018). Because of the increasing knowledge linking postsynaptic proteins to learning and memory, single postsynaptic proteins have garnered much attention as potential contributors to ASD etiology. Recently, several postsynaptic proteins, including *SynGAP*, appeared in a genome-wide analysis of ASD-linked copy number variations (Pinto et al., 2010), supporting the hypothesis that mutations leading to disrupted *SynGAP* function could result in behavioral phenotypes reminiscent of ASDs, including repetitive and obsessive behaviors and abnormal social interaction and communication (Parker et al., 2015; Mignot et al., 2016; Holder et al., 2019). The link between *SYNGAP1* disorder and ASDs was substantially strengthened by evidence suggesting that *SYNGAP1* haploinsufficiency causes premature maturation of dendritic spines (Clement et al., 2012), as dysregulation of spine maturation during development is thought to contribute greatly to the etiologies of other ASD-associated neurodevelopmental disorders, including Fragile X syndrome (He and Portera-Cailliau, 2013). *SYNGAP1*-related deficits in dendritic spine development cause not only a general aberration from the normal functioning of individual synapses, but also impairment of the development of the neural circuits they compose. This synapse-circuit duality is important to consider in the development of therapeutic strategies for *SYNGAP1* disorder.

Figure 5. Working model of SynGAP function at the synapse. Top left, SynGAP is enriched in the PSD through binding with MAGUK family scaffold proteins, such as PSD-95. NMDARs and AMPARs also interact with MAGUK proteins. The PSD is a phase-separated macromolecular complex that remains highly packed despite the lack of enclosure by lipid membranes. SynGAP inhibits the activity of Ras and other small GTPases, which are involved in numerous biochemical signaling pathways that promote enhancement of spine growth and synaptic strength through actin polymerization and AMPAR insertion. SynGAP also occupies a significant number of MAGUK PDZ domains, potentially placing a limit on the number of synaptic AMPAR/TARP complexes at the PSD. Top right, Following an LTP-inducing stimulus, SynGAP is rapidly dispersed from the PSD. This lifts the brake on small GTPase signaling, promoting plasticity-related biochemical and structural changes to the synapse. Some freely diffusing AMPAR/TARP complexes associate with MAGUK PDZ domains previously occupied by SynGAP. Bottom left, In the case of SynGAP haploinsufficiency, small GTPase signaling is basally elevated and MAGUK PDZ domains are basally more available for the binding of AMPAR/TARP complexes, resulting in larger spines with greater AMPAR content. SynGAP-haploinsufficiency-induced enhancement of basal synaptic AMPAR number represents basal enhancement of synaptic strength. Bottom right, Following a stimulus that would normally lead to robust activity-induced SynGAP dispersion, no further enhancement of synaptic strength is observed due to occlusion of LTP.

Schizophrenia

SYNGAP1 mutations have recently been suggested to play a role in the manifestation of other neurodevelopmental disorders, such as schizophrenia. One study found that elderly human patients with schizophrenia, on average, exhibit lower SynGAP expression levels in the anterior cingulate cortex (Funk et al., 2009), a brain region thought to be involved in a variety of high-level cognitive processes, including attention, decision making, and emotion (Bush et al., 2000). Some schizophrenia-related phenotypes have been observed in SynGAP heterozygous mice, including hyperactivity that was reduced by treatment with an antipsychotic drug, an increase in startle response with reduced prepulse inhibition, and a propensity for social isolation (Guo et al., 2009). Recently, the *SYNGAP1* gene was identified as a possible susceptibility risk gene for schizophrenia following annotation of schizophrenia risk loci from genome-wide association studies (Schizophrenia Working Group of the Psychiatric Genomics, 2014; Niu et al., 2019). From exome sequencing studies, *SYNGAP1* emerged as a component of complex gene interaction networks that might contribute to schizophrenia but did not rise to single-gene exome-wide significance (Purcell et al., 2014; Genovese et al., 2016). The mechanisms through which SYN-

GAP1 mutations might lead to schizophrenia-like symptoms are not clear.

A multifactorial role for SynGAP in neuronal function

Research over the last two decades has illuminated the critical importance of SynGAP for proper neuronal function, brain development, and cognition. In Figure 5, we present a working model of SynGAP function that includes the regulation of plasticity-associated signaling cascades as well as potential structural roles in regulating PSD composition. At the physiological level, roles for SynGAP have emerged not only in synaptic plasticity but also in neuronal development. It has been appreciated that the signaling functions of SynGAP are complex, as SynGAP differentially regulates multiple signaling cascades depending on its phosphorylation status. Structurally, it has been proposed that SynGAP dynamically occupies PSD binding “slots” and that changes in SynGAP localization during plasticity may increase the apparent affinity of other synaptic molecules, including AMPARs, for the PSD. In the cognitive domain, SynGAP has been shown to be specifically important for many higher-order processes, including memory and sensory processing. The requirement of SynGAP for normal cognition is especially high-

lighted by the neurological and intellectual deficits exhibited by individuals with *SYNGAP1* mutations. However, therapeutic strategies to treat *SYNGAP1*-related disorders are elusive beyond genetic approaches to restore SynGAP expression.

To begin to close the gaps in knowledge surrounding the central mechanisms underlying plasticity, learning, and *SYNGAP1*-related neurodevelopmental disorders, several key questions about SynGAP function must be answered:

1. To what extent is SynGAP function dependent on localization and isoform identity?

It has been challenging to convincingly dissect the contributions of individual SynGAP structural isoforms to neuronal development and plasticity. Their biochemical properties, as well as their developmental expression profiles and localization patterns remain incompletely understood. Differences in the C-terminal domains of SynGAP structural isoforms are likely crucial in determining subcellular localization patterns, especially the propensity with which they associate with PSD scaffolding molecules. Very little, however, is known about the role of SynGAP N-terminal structural variants in determining SynGAP expression and localization.

2. Does SynGAP exert a structural function at the synapse that is distinct from its signaling roles?

While recent studies have hinted at structural roles for SynGAP at the synapse (Walkup et al., 2016; Zeng et al., 2016), it will be important for future studies to uncouple the proposed structural roles of SynGAP from its signaling roles. This is not a trivial problem because the regulation of SynGAP localization and function is likely interwoven. For example, phosphorylation of SynGAP by CaMKII regulates SynGAP function twofold: it decreases the PSD enrichment of SynGAP (Yang et al., 2011; Araki et al., 2015; Walkup et al., 2016) while also altering its GAP activity (Oh et al., 2004; Dosemeci and Jaffe, 2010; Walkup et al., 2015). Because of this, it is unclear to what extent the inverse relationship between SynGAP PSD enrichment and synaptic AMPAR content is due to competition for PSD binding “slots.”

3. Do the biochemical and biophysical properties of SynGAP discovered *in vitro* hold true *in vivo*?

To date, most studies of SynGAP function at the level of the synapse, neuron, and circuit have been performed in *in vitro* or *ex vivo* preparations. With the advent of advanced techniques and genetic tools in recent years, it has been possible to begin to probe the functions of SynGAP in circuit development and function *in vivo* (Aceti et al., 2015; Michaelson et al., 2018). However, it is still unclear whether activity-dependent synaptic plasticity *in vivo* involves or requires SynGAP dispersion as demonstrated *in vitro*.

The answers to these questions will allow for a more complete physiologic view of the molecular underpinnings of synaptic plasticity, and will be crucial for identifying treatment strategies for *SYNGAP1*-related disorders. The study of SynGAP has presented a unique opportunity to strengthen the understanding of the biochemistry underlying information storage in the brain, and will continue to serve as a powerful case study and tool for understanding and treating neurodevelopmental disorders.

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