PERSPECTIVE

The role of postsynaptic density proteins in neural degeneration and regeneration

The structure, function and plasticity of excitatory glutamatergic synapses in the brain are significantly altered by neurodevelopmental and neurodegenerative disorders (Grabrucker et al., 2011; Fourie et al., 2014; Musardo et al., 2014; Lee et al., 2015), resulting in impairments in cognition, learning, memory, motor and sensory function. Reversing these synaptic deficits is a major challenge for neuroscience, and significant research has focussed on glutamate receptors as therapeutic targets but human trials have shown little success. Recently, attention has turned to proteins localized to the postsynaptic density (PSD) of excitatory synapses, as these proteins control the trafficking and targeting of glutamate receptors at synapses. Not surprisingly, PSD proteins have been shown to be altered in human and rodent models of neurological disorders, suggesting changes in their expression during the disease process (Grabrucker et al., 2011; Fourie et al., 2014; Musardo et al., 2014; Mei et al., 2016). However, the sheer number of these proteins, each with unique splice variations and developmental expression profiles, raises the possibility that targeted expression of specific PSD protein subfamilies within defined time windows has significant therapeutic potential. Indeed rescue of normal social behaviour has recently been observed in adulthood in an autism rodent model by re-expression of *SHANK3*, which encodes a major PSD protein, yet rescue of anxiety and motor coordination deficits required *SHANK3* re-expression earlier in development (Mei et al., 2016). Peptide disruption of PSD protein interactions with glutamate receptors is also showing promise to reduce excitotoxicity and ischaemic damage in stroke models (Zhou et al., 2015). Increasing our knowledge of the potential of these proteins in human neurons is the next important step, as this will be key to determining their true therapeutic potential for neuroregeneration.

PSD proteins: The PSD is a membrane specialization that extends across the top of the postsynaptic dendritic spine, directly opposing the presynaptic active zone. The PSD is approximately 200–800 nm wide and 30–50 nm thick, and is composed of postsynaptic receptors, cytoplasmic scaffold proteins, signalling enzymes and cytoskeletal structural elements (**Figure 1**). A major role of the PSD is to stabilize and anchor glutamate receptors such as alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors at synapses (Montgomery et al., 2004). In addition, PSD proteins also regulate the trafficking, targeting and insertion of receptors and ion channels along dendrites and into synapses.

Two major PSD protein families are membrane associated guanylate kinases (MAGUKs) and Src homology 3 (SH3) domain and ankyrin repeat proteins (Shanks), both of which have been shown to have major roles at excitatory glutamatergic synapses. Within the MAGUK family are the synaptic proteins PSD95, PSD93, SAP97, and SAP102, which share a common multi-domain structure with three PDZ domains, an SH3 domain, and a GUK domain through which they interact with AMPA and NMDA-type glutamate receptors and other ion channels (**Figure 1**; Montgomery et al., 2004). MAGUK proteins such as SAP97 interact with glutamate receptors early in the biosynthetic pathway in the endoplasmic reticulum, where they form trafficking complexes that transport receptors along dendritic microtubules, and then guide their insertion and stabilisation at synapses. PSD95 was the first major protein to be characterized in the PSD where it binds to the GluN2A/B subunit of the NMDA receptor at C-terminal regions. However, PSD95 is not a key regulator of NMDA receptors, but rather it plays a significant role in regulating the number of AMPA receptors at the synapse through interactions with the transmembrane AMPA receptor regulatory proteins (TARPs). Recent advances in super resolution microscopy have revealed that PSD95 forms distinct subdomains with AMPA receptors within the PSD that can be dynamically altered by synaptic activity. Other major PSD scaffold proteins that also form these subdomains include Shanks, Homer and GKAP (MacGillavry et al. 2013). These data suggest that the PSD is a dynamic structure that can re-model glutamate receptor clusters into distinct compartments. The functional roles and significance of these clusters are yet to be determined.

The Shank family of proteins (Shank1, Shank2, and Shank3) are characterized by a set of protein - protein interaction domains: ankyrin repeats, an Src homology 3 (SH3) domain, a PDZ domain, a proline-rich/homer and contact in binding domain and a sterile alpha motif (SAM) domain. Shank proteins are localized at the core of the PSD where they interact directly and indirectly with AMPA- and NMDA-type glutamate receptors, signalling molecules, and cytoskeletal actin to modulate synapse structure, and drive increases in synaptic strength and maturation (**Figure 1**). As such, they are often described as the "master regulators" of glutamatergic synapses as they form the focal point of the postsynaptic scaffold. Shanks can also signal trans-synaptically to alter the presynapse. Through interactions with the neurexin-neuroligin complex that spans the synaptic cleft, Shank3 drives increases in presynaptic protein expression and increases functional neurotransmitter release (Arons et al., 2012), further supporting the dominant and divergent roles that this protein family can exert on synapse function.

In addition to the large number of different proteins concentrated within the PSD, multiple spice variants exist for each protein, suggesting that each PSD protein has varying functional roles at synapses. For example, N-terminal splice isoforms in SAP97 have been shown to have opposite roles in regulating pools of synaptic and extrasynaptic AMPA receptors (Li et al., 2011), suggesting that this protein holds a range of functional responsibilities. Alternative splicing of Shanks also generates an array of mRNA and protein isoforms, resulting in proteins with varied domain compositions: In Shank1 splice variants, the N-terminal Ankyrin and SH3 domains, or the C-terminal SAM domain can be truncated. As a result, splicing alters protein-protein interactions that these domains mediate, and additionally alters spatiotemporal and subcellular localisation in the developing and adult brain (Lim et al., 1999). The extent of the functional effects different PSD protein splice isoforms have at synapses is still far from understood, but it is clear that splicing adds further diversity to their roles in regulating synapse structure, transmission and plasticity.

PSD proteins and neurological disorders: The prominent roles that the PSD scaffolding proteins play in glutamate receptor trafficking and scaffolding suggest that perturbations to these proteins underlie the synaptic pathology of neurological developmental and degenerative diseases. The subcellular distribution and expression levels of SAP97, PSD93, PSD95, SAP102 and Shanks have been observed to be significantly altered in rodent models of Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD), and also in postmortem human AD, PD and/or HD brain (Grabrucker et al., 2011; Fourie et al., 2014). Abnormal PSD protein-protein interactions are also thought to contribute to glutamatergic synapse dysfunction in neurodegenerative diseases such as AD, where SAP97 interactions with ADAM10 (A disintegrin and metalloproteinase 10) are disrupted to favour amyloid protein plaque formation (Musardo et al., 2014).

While PSD proteins are clearly affected by the disease process, their wide range of functional roles, splice variations and expression profiles provide valuable opportunities to play a role in brain repair. As pharmacological targeting of glutamate receptors has not proven fruitful in the treatment of neurological disorders, recent research has turned to PSD proteins to determine their potential as therapeutic targets to alter glutamatergic synapses. Modulation of PSD protein interactions with their binding partners using small cell permeable peptides is a strategy that is showing strong therapeutic promise without significant side effects. For example, peptide disruption of PSD95 interactions with neuronal nitric oxide synthase (nNOS) or with the NMDA receptor results in decreased excitotoxic and ischaemic damage in stroke models (Zhou et al., 2015). With regards to neurodegenerative diseases, peptides that uncouple GluN2A binding to PSD-MAGUKs significantly reduced dyskinesias in PD rodent models (Gardoni et al., 2012).

An alternative strategy to rescue synaptic function that is altered by disease processes is specifically altering the synaptic expression of PSD

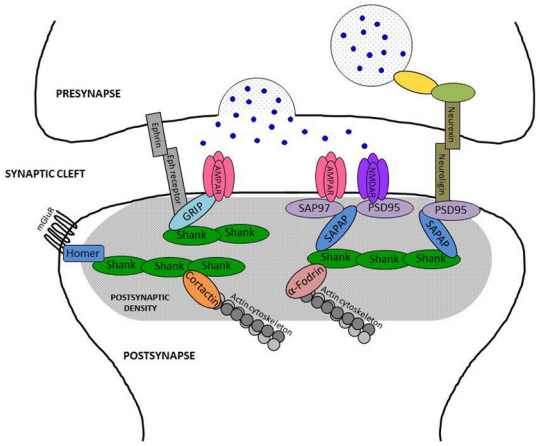

Figure 1 Postsynaptic density (PSD) proteins at excitatory synapses in the brain.
The PSD is located directly opposite the presynaptic active zone where glutamate is released. Postsynaptic glutamatergic receptors such as alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors are localized at the postsynaptic membrane where they detect synaptically released glutamate. The targeting, surface expression, localization and removal of glutamatergic receptors is regulated by interactions with PSD proteins including the membrane associated guanylate kinases (MAGUKs) PSD95 and SAP97, and other multidomain proteins such as glutamate receptor interacting protein (GRIP). Shank proteins form multimeric sheets within the PSD where they interact with numerous PSD proteins and with the actin cytoskeleton. Shank interactions with the trans-synaptic bridge formed by neurexin and neuroligin also enables Shank-dependent regulation of presynaptic structure and function (Arons et al., 2012).

proteins. An example of this is the regulation of Shank protein expression by zinc supplementation in AD (Grabrucker et al., 2011). A role for zinc in AD was first suggested many years ago, and because the organization of postsynaptic Shank platforms at the PSD is regulated by zinc, resulting in increased postsynaptic structural integrity, it is proposed that amyloid-beta binds synaptic zinc so it is unable to bind Shank proteins. This disrupts the Shank platform across the PSD, significantly decreasing glutamatergic synapse number and function. Treatment of hippocampal neurons with zinc was found to rescue synaptic Shank3 expression and bring synapse density to normal levels (Grabrucker et al., 2011).

PSD proteins are also emerging as major contributors to not only neurodegenerative but also to neurodevelopmental disorders. For example, mutations in the Shank family of proteins have been identified in multiple neurodevelopmental disorders including autism spectrum disorders (ASD), Phelan-McDermid syndrome, and schizophrenia. Considering the fundamental role played by the Shank proteins in coordinating the function and maturation of excitatory synapses in the brain, it is not surprising that mutations in *SHANK* significantly impair glutamatergic synaptic structure and function (Arons et al., 2012). Multiple animal models of ASD have been created and reductions in the expression levels of PSD proteins, glutamate receptors, synaptic transmission, synaptic plasticity, postsynaptic spine structure and density were reported in these animals. Behavioural testing of these ASD rodent models also revealed deficits in social interactions, as well as increases in repetitive behaviours and also anxiety (Lee et al., 2015; Mei et al., 2016).

How PSD proteins regulate social and cognitive behavior is beginning to be identified, highlighting these pathways as promising candidates in the development of therapeutic targets. For example, in two independent ASD mouse models, one lacking Shank2 and another with haploinsufficiency in the neural-specific transcription factor Tbr1, elevating postsynaptic zinc levels with clioquinol resulted in the rapid rescue of social interaction behaviours (Lee et al., 2015). This occurred via restoration of NMDA receptor function by postsynaptic activation of the tyrosine kinase Src (Lee et al., 2015). In Shank3 ASD mutant mice that show glutamatergic synaptic deficits and ASD-related behavioural impairments in social interaction, anxiety and repetitive behaviours, re-expressing the Shank3 gene in adult mice improved synaptic deficits, social interaction and repetitive grooming (Mei et al., 2016). Intriguingly, the timing of re-expressing *SHANK3* is critical, as anxiety and motor coordination deficits were not recovered in adults, but could be rescued by *SHANK3* re-expression during development (Mei et al., 2016). This work highlights the therapeutic potential of PSD proteins, even in the mature brain, however the developmental expression profiles that different PSD proteins display, and their gene regulation by splicing and epigenetic factors such as DNA methylation or histone acetylation may create "therapeutic windows" in their efficacy.

Future directions: PSD proteins clearly have significant regulatory control over glutamatergic synapses, and may be feasible new pharmaceutical targets in the development of novel therapies. However, current biomedical research is limited with respect to detailed knowledge of these proteins in human neurons, and whether their functional roles are directly comparable to animal studies. This is challenging to determine in primary human neurons for obvious reasons, but could be readily examined in induced human neuronal cells where knowledge of their synaptic physiology is still developing. This information will be key in developing therapies to restore the synaptopathology that occurs with almost every neurological disorder.

Yukti Vyas, Johanna M. Montgomery*

Department of Physiology and Centre for Brain Research, University of Auckland, Auckland, New Zealand

***Correspondence to:** *Johanna M. Montgomery, Ph.D., jm.montgomery@auckland.ac.nz.*
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