A Role for Synaptic Zinc in ProSAP/Shank PSD Scaffold Malformation in Autism Spectrum Disorders

Andreas M. Grabrucker[1,2]

[1] Neurology Department, WG Molecular Analysis of Synaptopathies, Neurocenter of Ulm University, Ulm, Germany

[2] Institute for Anatomy and Cell Biology, Ulm University, Ulm, Germany

Received 10 September 2012; revised 27 March 2013; accepted 25 April 2013

ABSTRACT: The establishment and maintenance of synaptic contacts as well as synaptic plasticity are crucial factors for normal brain function. The functional properties of a synapse are largely dependent on the molecular setup of synaptic proteins. Multidomain proteins of the ProSAP/Shank family act as major organizing scaffolding elements of the postsynaptic density (PSD). Interestingly, ProSAP/Shank proteins at glutamatergic synapses have been linked to a variety of Autism Spectrum Disorders (ASDs) including Phelan McDermid Syndrome, and deregulation of ProSAP/Shank has been reported in Alzheimer's disease. Although the precise molecular mechanism of the dysfunction of these proteins remains unclear, an emerging model is that mutations or deletions impair neuronal circuitry by disrupting the formation, plasticity and maturation of glutamatergic synapses. Several PSD proteins associated with ASDs are part of a complex centered around ProSAP/Shank proteins and many ProSAP/Shank interaction partners play a role in signaling within dendritic spines. Interfering with any one of the members of this signaling complex might change the output and drive the system towards synaptic dysfunction. Based on recent data, it is possible that the concerted action of ProSAP/Shank and Zn^{2+} is essential for the structural integrity of the PSD. This interplay might regulate postsynaptic receptor composition, but also transsynaptic signaling. It might be possible that environmental factors like nutritional Zn^{2+} status or metal ion homeostasis in general intersect with this distinct pathway centered around ProSAP/Shank proteins and the deregulation of any of these two factors may lead to ASDs. © 2013 The Authors. Developmental Neurobiology Published by Wiley Periodicals, Inc. Develop Neurobiol 74: 136–146, 2014

Keywords: **Shank3; Shank2; Phelan McDermid Syndrome; 22q13; Zn^{2+}**

PROSAP/SHANK AND ASDS

The loss of one copy of ProSAP2/Shank3 in humans contributes significantly to autistic behavior in Phelan McDermid Syndrome (22q13 deletion syndrome) (Bonaglia et al., 2001 ; Phelan et al., 2001; Wilson et al., 2003; Manning et al., 2004). Most cases of Phelan McDermid Syndrome are due to *de novo* breaks in the long arm of chromosome 22 causing a microdeletion of chromosome 22 in which a portion of the distal long arm (q) of the chromosome is missing (Wong et al., 1995). The syndrome is characterized by moderate to profound mental retardation, neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech (Manning et al., 2004), and minor dysmorphic features (Phelan, 2008). The behavior is described as "autistic-like" with tactile defensiveness, anxiety in social situations, avoidance of eye contact, and self-stimulatory behavior. Based on limited statistical

Correspondence to: A.M. Grabrucker (andreas.grabrucker@uni-ulm.de).

Published online 4 May 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/dneu.22089

analysis, the occurrence rate has been estimated to fall in the range 2.5–10 per million births (Trabacca et al., 2011). This rate will likely increase with the improvement of genetic tools, since so far, many individuals with this deletion have required two or more chromosome studies before the deletion was detected (Phelan et al., 2001). At present, the treatment for Phelan McDermid Syndrome is symptomatic and supportive and addresses the individual symptoms of each patient. The haploinsufficiency of the ProSAP2/Shank3 gene seems to provide the most direct link of a ProSAP/Shank protein family member to an ASD. Additionally, mutations associated with autism have recently been reported in ProSAP1/Shank2, ProSAP2/Shank3, and Shank1 (Durand et al., 2007; Moessner et al., 2007; Gauthier et al., 2009; Berkel et al., 2010; Pinto et al., 2010; Sato et al., 2012).

Mutations in ProSAP2/Shank3 linked to ASD were the first to be found in a ProSAP/Shank family member and included deletions, duplications, and point mutations (Durand et al., 2007; Moessner et al., 2007; Gauthier et al., 2009). Subsequently, mutations associated with ASD were identified in ProSAP1/Shank2 (Berkel et al., 2010; Pinto et al., 2010) and more recently in Shank1 (Sato et al., 2012). Intriguingly, mutations in ProSAP1/Shank2 (C1384T), as well as ProSAP2/Shank3 (InsG3680) were found that both might affect its ability to localize synaptically due to the disruption of the ProSAP/Shank C-terminal Zn^{2+}-binding SAM domain. However, ProSAP/Shank proteins are not the sole genes to be directly associated with ASD. Genomic mutations, deletions or duplications associated with ASDs have been found in multiple genes. A surprising number of these mutations can be placed in a hypothetical pathway at excitatory glutamatergic synapses, where scaffold proteins like ProSAP1/Shank2, ProSAP2/Shank3, Shank1, GKAP, or LASP1 can be found at the postsynaptic density (PSD) (Grabrucker et al., 2011a). Moreover, ProSAP/Shank proteins bind trans-synaptic complexes of Neuroligins and Neurexins (Meyer et al., 2004) that are cell adhesion molecules which coordinate post- and pre-synaptic structural plasticity (Arons et al., 2012). They also cluster receptors at the PSD such as group I mGluRs (Verpelli et al., 2011), which are influenced by FMRP (fragile X mental retardation protein) (Hagerman et al., 2010). Mutations/deletions in every protein mentioned above have already been associated with ASDs. Nevertheless, it is possible that most of the identified genes associated with ASDs are part of a common mechanism that is not only influenced by mutations or deletions of the genes, but also by the environment (e.g. metal ion homeostasis) in which the encoded proteins function.

PROSAP/SHANK AND ZINC

Chemical synapses serve as highly advanced cell junctions in the body, which retain a remarkable plasticity throughout their lifetime. This synaptic plasticity determines the strength of synaptic transmission based on the past activity of a synapse. This strength can be modulated by either presynaptic or postsynaptic mechanisms and can last from a few hundred milliseconds to weeks or even longer. Short-term plasticity mechanisms are predominantly mediated through local modifications of proteins, while long-term plasticity leads to structural changes in synaptic contacts and is dependent on *de novo* protein synthesis (Lynch, 2004). Synaptic plasticity is considered as the basis for all key functions of the brain, including the processes of learning and memory.

Excitatory glutamatergic synapses are characterized by a particularly complex postsynaptic cytomatrix, the PSD. The PSD is a network of proteins that together detect, transduce and integrate synaptic signals. Given that ProSAP/Shank proteins occupy a central position in the PSD and have a large variety of binding partners, they can be considered to be master scaffolding proteins of the PSD (Boeckers et al., 2002). Previous studies demonstrated that the concerted action of ProSAP/Shank and zinc ions is essential for the structural integrity of the PSD (Grabrucker et al., 2011b).

Within the brain Zn^{2+} plays a role in synaptic transmission, serves as an endogenous neuromodulator and is important for nucleic acid metabolism and brain microtubule growth (Pfeiffer and Braverman, 1982). Zinc ions are enriched in presynaptic vesicles of glutamatergic terminals (Frederickson et al., 2005) and Zn^{2+} that is released from the nerve terminal binds to Zn^{2+} receptors and glutamate-receptors and/or enters the postsynaptic compartment (Assaf et al., 1984; Howell et al., 1984; Li et al., 2001; Fredericksen et al., 2005; Huang et al., 2008; Besser et al., 2009). Intracellularly, Zn^{2+} is found almost exclusively bound to proteins. Jan et al. (2002) have shown that the Zn^{2+} content of purified PSDs is relatively high with 4.1 nmoles/mg protein and that *in vitro* disassembled PSDs can be reassembled following Zn^{2+} binding to certain Zn^{2+}-binding proteins (Jan et al., 2002) possibly including ProSAPs/Shanks that are a critical part of the PSD.

ProSAP/Shank proteins (ProSAP1/Shank2 and ProSAP2/Shank3) possess a C-terminal sterile-alpha-motif (SAM) domain that is able to bind a Zn^{2+}.

Currently, more than 100 different SAM domains have been identified in mammalian genomes (Gundelfinger et al., 2006). However, not every SAM domain is able to bind Zn^{2+} (Qiao and Bowie, 2005) and SAM domains have multiple functions. One feature for instance is the ability to self-associate, forming either oligomers or homo- or hetero-polymers or in some instances binding to RNA and DNA (Qiao etal., 2004; De Rycker and Price, 2004; Song et al., 2005; Aviv et al., 2006; Oberstrass et al., 2006; Qiao et al., 2006). In line with this, the ProSAP2/Shank3 SAM domain is able to self-associate (Naisbitt et al., 1999) and may engender structural and functional heterogeneity by the incorporation of different ProSAPs/Shanks into heteropolymers. The ProSAP2/Shank3 SAM domain and the ProSAP1/Shank2 SAM domain are necessary for synaptic localization of the protein (Boeckers et al., 2005), however, the role of the Shank1 SAM domain is less clear. The sequence similarity among the SAM domains of the ProSAP/Shank family members is very high with ProSAP2/Shank3 sharing 72% and 68% identity with Shank1 and ProSAP1/Shank2, respectively (Gundelfinger et al., 2006). However, a leucine residue in ProSAP2/Shank3 (Leu1742) is changed to a phenylalanine in Shank1. This amino acid is critical for the formation of sheets since mutation of Leu1742 to alanine in ProSAP2/Shank3 interferes with ProSAP2/Shank3 sheet formation. In contrast to ProSAP2/Shank3 and ProSAP1/Shank2, the Shank1 SAM domain is not required for localization of Shank1 to synapses (Boeckers et al., 2005) and it is probably only sufficiently targeted to PSDs with a preformed ProSAP2/Shank3 and ProSAP1/Shank2 scaffold (Grabrucker et al., 2011b).

The ProSAP2/Shank3 SAM domain forms a helical polymer and, with the ability of ProSAP2/Shank3 SAM domain polymers to align side by side, can form a sheet-like structure (Baron et al., 2006). The ProSAP2/Shank3 sheet therefore can be found as a plane of helical fibers that leave the remaining domains of ProSAP2/Shank3 unaffected. In this manner, assembled sheets of ProSAP2/Shank3 are able to interact with both PSD proteins and cytoskeletal components.

Zn^{2+} binding regulates the packing density of the ProSAP/Shank within sheets (Baron et al., 2006) with Zn^{2+} ions being recruited into large macromolecular platforms assembled by the SAM domains (Qiao and Bowie, 2005) of ProSAP2/Shank3 and potentially ProSAP1/Shank2 (Baron et al., 2006; Gundelfinger et al., 2006). The Zn^{2+}-binding site in the ProSAP/Shank SAM domain is located in a way that assists sheet formation. In particular, Zn^{2+} seems to stabilize salt bridges across two SAM domain interfaces by directly binding one amino acid in each pair (Baron et al., 2006). Two of the three ProSAP/Shank family members are able to effectively bind Zn^{2+} via their SAM domains. Shank1 seems to stabilize synapses in a Zn^{2+} insensitive mechanism. Since nascent PSDs appear to initially possess only ProSAP1/Shank2 and ProSAP2/Shank3 (Boeckers et al., 1999; Grabrucker et al., 2011b), it is possible that initial contacts can only be maintained in the presence of a sufficient local Zn^{2+} concentration. Furthermore the artificial addition of Zn^{2+} leads to increased clustering and targeting of ProSAP1/Shank2 and ProSAP2/Shank3, which suggests a possible mechanism to alter local ProSAP/Shank protein levels (Grabrucker et al., 2013). Keeping in mind that Zn^{2+} is released with synaptic transmission, it is intriguing that the ProSAP2/Shank3 sheets may quickly change shape by assembly or disassembly of the sheet from smaller units as a result of adding or removing SAM domain interactions (Gundelfinger et al., 2006) driven by the local Zn^{2+} concentration. In line with this, previous experiments have shown that cells growing in Zn^{2+} — depleted medium have less synaptic ProSAP1/Shank2 and ProSAP2/Shank3 immunoreactive puncta per 10 μm dendrite length at DIV10 using immunocytochemistry. CaEDTA and TPEN [N,N,N′,N′-*tetrakis*-(2-Pyridylmethyl)ethylenediamine], two potent Zn^{2+} chelators, trigger redistribution of ProSAP2/Shank3 and ProSAP1/Shank2 from the synapse to a more diffuse and dendritic localization in hippocampal neurons in culture (Grabrucker et al., 2011b).

ProSAP/Shank sheets within the PSD function as docking sites for a large number of interacting proteins. Thus it is reasonable that additional ProSAP/Shank proteins may provide new docking sites and influence the overall structure of the PSD. We previously proposed a model in which ProSAP/Shank protein expression as well as the amount of ProSAP/Shank protein targeting to excitatory postsynapses has an impact on proper synapse establishment and maturation (Grabrucker et al., 2011a). This, in turn, may lead to further downstream effects influencing the number of interaction partners including Neuroligin/Neurexins (Arons et al., 2012). Since Neurexin and Neuroligin interact across the synaptic cleft this has the potential to transmit changes in the PSD to the presynaptic site. On the other hand, a down-regulation of ProSAP/Shank, as found in Phelan McDermid Syndrome, or Alzheimer's disease patients, might lead to a loss of sufficient scaffold molecules, affecting the overall number and stability of the PSD. The pathways through which these effects might

unfold are still not well understood, but the connection to Neuroligin/Neurexin transsynaptic signaling might, again, be an important factor. A Neurexin/Neuroligin/Shank signaling pathway (Bourgeron, 2009) could not only be important to stabilize synaptic contacts, but also to mediate synaptic plasticity and maturation.

The availability for additional binding sites for exocytosed AMPAR might thus be dependent on activity- and Zn^{2+}-dependent increases in ProSAP2/Shank3 scaffold proteins at the PSD. Conversely, a decrease in AMPA receptor levels was shown in ProSAP2/Shank3$\alpha\beta$–/– mice (Bozdagi et al., 2011). Additionally, the upregulation of ProSAP2/Shank3 protein levels at the PSD via Zn^{2+} causes a transsynaptic signal. Recent data shows that an increase in ProSAP2/Shank3 leads to an increase in presynaptic protein levels and that this signal can be uncoupled blocking Neuroligin–Neurexin complex formation (Arons et al., 2012). Moreover, the increase in postsynaptic ProSAP2/Shank3 also increased the vesicle pool in the terminal (Arons et al., 2012). Thus, taken together, the regulation of ProSAP2/Shank3 via Zn^{2+} is at the center of two crucial features of synaptic activity dependent plasticity—the increase in postsynaptic receptor density as basis for LTP, and the coordination of presynaptic plasticity along with postsynaptic changes.

Based on this, a model can be proposed (Fig. 1), wherein synaptic activity releases Zn^{2+} into the synaptic cleft, as well as activating NMDA and AMPA receptors. Zn^{2+} may then translocate into the postsynapse and bind ProSAP2/Shank3 proteins, that are part of a soluble pool, shifting some molecules to the PSD bound pool and thus stabilizing or remodeling the ProSAP2/Shank3 PSD scaffold. Alternatively, it is possible that synaptic activity releases Zn^{2+} from postsynaptic stores such as mitochondria or from metallothioneins (MTs) to which it is bound. Mice lacking the vesicular Zn^{2+} transporter have reduced presynaptic stores of Zn^{2+}. Initial observations revealed no synaptic or behavioral deficits (Cole

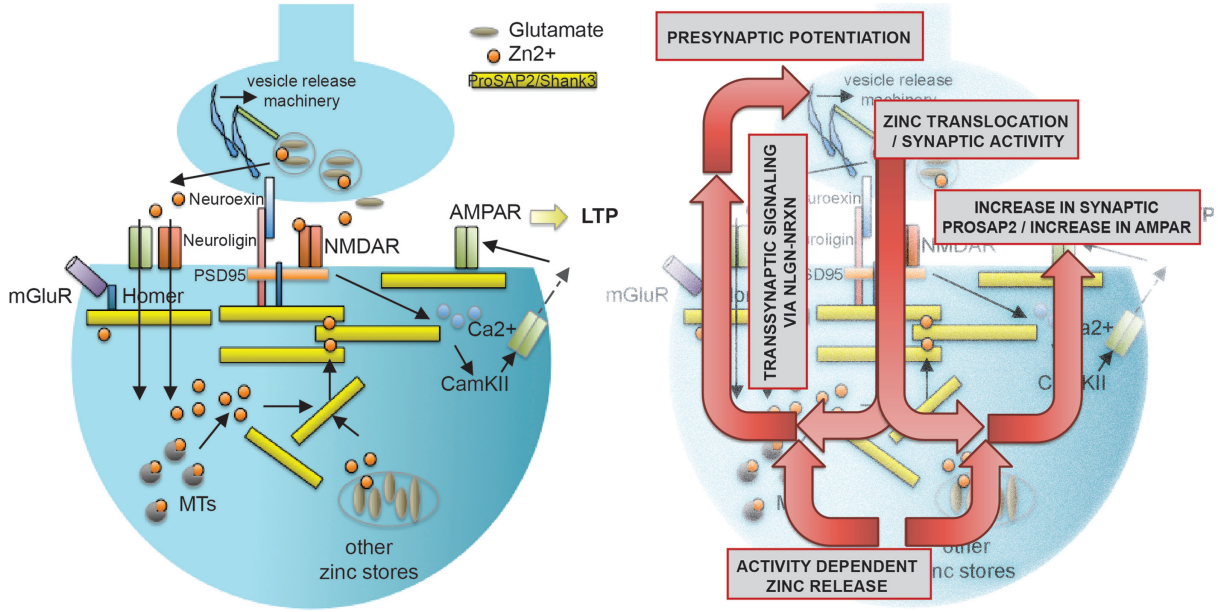

Figure 1 A model for postsynaptic Zn^{2+} signaling via ProSAP2/Shank3 at glutamatergic synapses. (Left) Synaptic activity leads to glutamate and Zn^{2+} release from the presynaptic terminal followed by activation of postsynaptic NMDA and AMPA receptors. Along with this, free Zn^{2+} enters into the postsynaptic compartment via channels such as NMDAR or AMPAR. However, synaptic activity might also release Zn^{2+} from MTs or other Zn^{2+} stores. This Zn^{2+} is able to bind ProSAP2/Shank3 proteins that are part of a soluble pool shifting some molecules to the PSD bound pool and thus stabilizing the ProSAP2/Shank3 PSD scaffold. In parallel, as a mechanism of LTP after synaptic activity and for instance mediated by the increase in postsynaptic Ca^{2+} and the activation of CamKII, additional AMPARs might be trafficked to the postsynaptic membrane and anchored to the PSD via newly attached ProSAP2/Shank3 PSD scaffold proteins. (Right) An increase in postsynaptic size must be coordinated with presynaptic changes. Intriguingly, the upregulation of ProSAP2/Shank3 protein levels at the PSD leads to an increase in presynaptic protein and vesicle levels mediated by Neuroligin–Neurexin complexes.

et al., 1999) in these mice but several subsequent studies have shown that they have deficits in contextual discrimination and spatial working memory (Adlard et al., 2010; Martel et al., 2010; Sindreu et al., 2011). Mice lacking the neuronally expressed MT-3 in contrast show a reduction in brain Zn^{2+} concentrations due to the absence of Zn^{2+} bound to MT-3 leaving the presynaptic stores unaffected (Erickson et al., 1995, 1997). They display diminished social interactions, mimicking aspects of ASDs, (Koumura et al., 2009) as well as defects in synaptic transmission. Thus, presynaptically released Zn^{2+} (Fig. 1), but more likely postsynaptically released Zn^{2+} or both may contribute to ProSAP/Shank regulation of glutamatergic synapses and the circuits in which these synapses are critical.

However, each ProSAP/Shank family member might contribute to makeup and plasticity of the synapse in its own specific way. For example, the overexpression of Shank1 induces maturation of dendritic spines without increasing synapse density, whereas overexpression of ProSAP2/Shank3 induces the formation of new synapses and even dendritic spines (Roussignol et al., 2005; Grabrucker et al., 2011b). Moreover, Shank1, that does not bind Zn^{2+} and that depends on its PDZ domain rather than its SAM domain for synaptic localization, forms a complex with Homer in the PSD (Hayashi et al., 2009). Although both ProSAP1/Shank2 and ProSAP2/Shank3 may localize to synapses in a Zn^{2+} dependent manner, they might play different roles within the PSD. For example, within the striatum, ProSAP1/Shank2 knockout mice partially compensate for the absence of ProSAP1/Shank2 with ProSAP2/Shank3, and ProSAP2/Shank3$\alpha\beta$ knockout mice show a compensation for the loss of ProSAP2/Shank3 by ProSAP1/Shank2 (Schmeisser et al., 2012). However, these animals still show specific deficits in the glutamate receptor composition with an increase in NMDA and AMPA receptor levels in ProSAP1/Shank2–/– and a decrease in AMPA receptor levels in ProSAP2/Shank3$\alpha\beta$–/– mice (Schmeisser et al., 2012). Although it may not be the sole factor for this compensation, one might speculate that ProSAP1/Shank2 and ProSAP2/Shank3 proteins form heteropolymers upon binding to Zn^{2+} at the PSD in widtype animals. In knockout animals however, homopolymers will be favored assuming equal Zn^{2+} concentrations in knockout and wildtype animals. Thus a net increase of the remaining family member is expected. However, the persistent alterations in receptor composition might indicate that homopolymeric ProSAP2/Shank3 scaffolds have a higher capacity to cluster glutamate receptors compared to homopolymeric ProSAP1/Shank2 scaffolds. Given the importance of the regulation of ProSAP2/Shank3 by Zn^{2+}, its deficiency might impact ProSAP/Shank scaffolds leading to synaptic defects.

ZINC AND ASDS

Previous studies have revealed a significantly increased incidence of Zn^{2+} deficiency in autistic patients compared to controls (Walsh et al., 2001, 2002; Jen and Yan, 2010; Yasuda et al., 2011) as well as an elevation of Cu^{2+} in samples of subjects with autism (Lakshmi Priya and Geetha, 2011). Moreover, further studies underlined that the Cu^{2+}/Zn^{2+} ratio is increased in subjects with autism (Faber et al., 2009). Cu^{2+} and Zn^{2+} have competing roles within the body with Cu^{2+} overload causing Zn^{2+} deficiency (Hall et al., 1979; Huster 2010) and the Cu^{2+}/Zn^{2+} ratio has been proposed as a biomarker for children with autism (Faber et al., 2009). However, based on recent data, Zn^{2+} deficiency—especially maternal Zn^{2+} deficiency during pregnancy - might not only be a biomarker for autism, but a risk factor. In a recent study, hair Zn^{2+} concentrations from 1967 children with autism were investigated and an incidence of Zn^{2+} deficiency in the infant-group aged 0–3 year-old was found with 43.5 % in males and 52.5 % in females (Yasuda et al., 2011): dramatically higher than the <1 % in the normal population. Taken together, these findings suggest that infantile Zn^{2+} deficiency may contribute to the pathogenesis of autism (Yasuda et al., 2011). Intriguingly, much evidence has accumulated that Zn^{2+} deficiency causes neuropsychological symptoms, learning and memory impairments (Golub et al., 1995), and behavioral and emotional problems in animal models and human subjects (Shankar and Prasad, 1998). Moreover, Zn^{2+} deficiency leads to an enhancement of glutamate excitotoxicity and is therefore associated with the occurrence of seizures. Interestingly, many patients with autism suffer from epilepsy (Tuchman and Cuccaro, 2011). It remains to be seen to what extent ProSAP/Shank function is a target of Zn^{2+} deficiency, although the affects on glutamatergic synapses are consistent with this notion.

In the telencephalon, all presynaptic boutons containing vesicular Zn^{2+} establish glutamatergic synapses that typically involve dendritic spines (Pérez-Clausell and Danscher, 1985). However, not every brain region contains "zincergic" synapses. Interestingly, neuronal projections from or to subcortical structures are largely devoid of presynaptic Zn^{2+}. Instead, zincergic projections selectively link

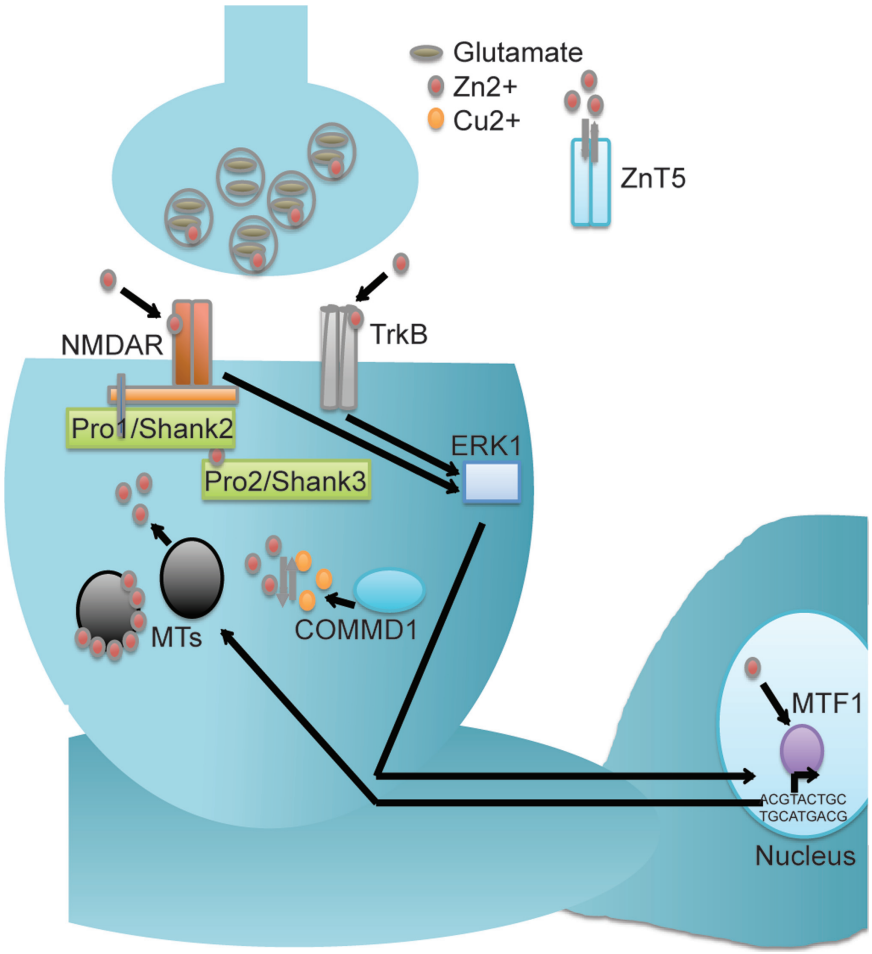

Figure 2 Candidate genes influenced by Zn^{2+} or involved in Zn^{2+} signaling and metal ion homeostasis associated with the development of ASDs. In the extracellular space, receptors like NMDAR and TrkB bind Zn^{2+}. Intracellular Zn^{2+} is mostly bound to metal-binding proteins of which the metallothionein family (MTs) is the most abundant. MTs are cysteine-containing proteins with high affinity for Zn^{2+}. Additionally, Zn^{2+} can associate with the SAM domains of ProSAP1/Shank2 and ProSAP2/Shank3. Downstream signals, including the Erk1/2 MAP kinase pathway, are activated by Zn^{2+} and Zn^{2+} is an essential co-factor for MTF-1, a transcription factor binding to metal response elements in the promoter region of genes. COMMD1 and ZnT5 might have a role in the general regulation of Zn^{2+} levels, given that ZnT5 is a Zn^{2+} transporter expressed in enterocytes and COMMD1 influences the intracellular Cu^{2+} which, in turn, affects Zn^{2+} homeostasis.

cortical and limbic structures (Sindreu and Storm, 2011). This suggests that zincergic neurons may be part of a subnetwork of projections found within the cortical system. Moreover, not every synapse within brain areas containing zincergic synapses has vesicular Zn^{2+}. Measurements in the CA1 region of the hippocampus indicate that about 50 % of Schaffer-collateral synapses may be zincergic (Sindreu et al., 2003). However, the hippocampal mossy fibers are an exception, since all mature dentate gyrus granule cells appear to establish zincergic terminals. Within the developing striatum, Zn^{2+} appears in striatal patches innervated by so called dopamine island fibers (Vincent and Semba, 1988). This rather selective distribution of zincergic synapses when correlated with behavioral deficits in mouse models may reveal interesting information about putative pathomechanisms of ASDs.

Intriguingly, several candidate genes that are associated with the development of ASDs, such as metallothioneins (MTs), ZnT5, ERK1, COMMD1, MTF1 (metal responsive transcription factor-1), TrkB, ProSAP1/Shank2, and ProSAP2/Shank3 (Serajee et al., 2004; Huang et al., 2008; Levy et al., 2011; O'Roak et al., 2011; Grabrucker et al., 2011a; Nuttall and Oteiza, 2012; Sanders et al., 2012) are influenced by

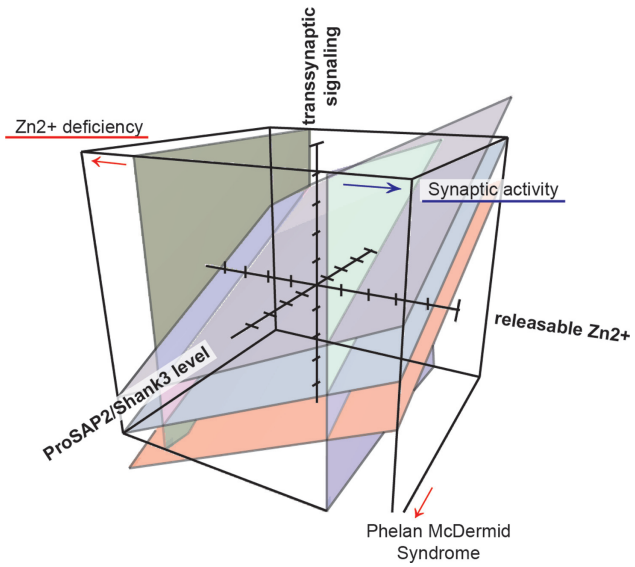

Figure 3 Transsynaptic signaling is determined by the amount of ProSAP2/Shank3 at the postsynaptic spine (ProSAP2/Shank3 in a soluble pool and PSD bound pool, here defined as concentration (C) of ProSAP2/Shank3 (Pr2/SH3): $C_{Pr2/SH3}$) and by the releasable pool of Zn^{2+} (concentration (C) of Zn^{2+}-ions (Zn): C_{Zn}) determining the increase of ProSAP2/Shank3 after synaptic activity. Assigning values of 1 to 20 to $C_{Pr2/SH3}$ and C_{Zn} with 10 being the "normal" state of a synapse, with transsynaptic signaling = $(C_{Zn} + C_{Pr2/SH3})/2$ one can calculate the resulting signal. Shown are two examples, one of synaptic activity = C_{Zn} +3 and one with Zn^{2+} deficiency = C_{Zn} -3. Phelan McDermid Syndrome in turn decreases $C_{Pr2/SH3}$.

Zn^{2+} or involved in Zn^{2+} signaling and metal ion homeostasis (Grabrucker, 2012), and might play a role at zincergic synapses (Fig. 2). For instance, Zn^{2+} is an essential co-factor for MTF-1, a transcription factor that binds to metal response elements in the promoter region of MT genes mediating their response to Zn^{2+} (Andrews, 2001; Wimmer et al., 2005). MTs are cysteine-containing, intracellular proteins with high affinity for Zn^{2+} and other metals (Andrews, 2000). However, Zn^{2+} is a highly efficient cofactor regulating MT expression (Park et al., 2001) enhancing MT-3 production in the hippocampus, amygdala and pyriform cortex (Faber et al., 2009) and increasing levels of vesicular Zn^{2+}. The transmission of a single nucleotide polymorphism (SNP) of the MTF1 gene was found to be associated with autism (Serajee et al., 2004). Given the importance of MTF1 for the regulation of MTs, mutations in MTF1 may adversely affect the utilization of Zn^{2+} stores within synapses. Moreover, Zn^{2+} activates TrkB by an activity-regulated mechanism at the PSD of excitatory synapses (Huang et al., 2008). Molecules downstream of TrkB, including the Erk1/2 MAP kinase pathway, are similarly activated by Zn^{2+} in cultures.

Zn^{2+} gets absorbed in the gastrointestinal tract. However, how circulating Zn^{2+} crosses the blood–brain barrier and enters the brain or CSF is not well understood, although active transport is likely. Also uptake of Zn^{2+} into the blood is important and might be compromised in some cases. ZnT5 (Wang and Zhou, 2010) is one of several Zn^{2+} transporters in enterocytes and other tissues (Jackson et al., 2008). ZnT5 has been associated with autism. The transporter might be involved in both, uptake and efflux of Zn^{2+} where mutations might contribute to a general deregulation of Zn^{2+} homeostasis affecting brain function. Zn^{2+} deficiency may occur *in utero* from maternal malnutrition or from increased exposure to heavy metals such as copper. *In utero*, mammals that have decreased levels of MTs may use up Zn^{2+} reserves faster compared to fetuses with normal MT function (Faber et al., 2009). Thus, taken together, a transient Zn^{2+} deficiency in a critical time window of development might be able to modify a synaptic pathway associated with autism and influenced by Zn^{2+}. Indeed, Zn^{2+} deficiency in pregnant rhesus monkeys has effects on the social behavior of infants (Sandstead et al., 1978).

CONCLUSIONS

Despite shared characteristics like abnormal social behavior, communication deficits, and repetitive or stereotyped behaviors, a great deal of heterogeneity

exists among ASD patients suggesting a complex etiology. Recent studies point to a strong genetic contribution to ASDs and mutations/deletion/duplications associated with ASD have been found in multiple genes (Li et al., 2012). Unfortunately, our information on how these genes function on a molecular level is incomplete and leaves open how their disturbance causes ASDs. On the other hand, environmental factors have been long discussed to play a role in ASD, either as cause or modifiers. For example, Zn^{2+} deficiency has been discussed extensively as risk factor for ASDs (Curtis and Patel, 2008; Grabrucker, 2012). Thus, it is increasingly likely that there are well defined pathways associated with the development of ASD and that these pathways can be influenced by the environment. ProSAP2/Shank3 might be at the nexus between environmental and genetic makeup. ProSAP2/Shank3 is heavily influenced by the local Zn^{2+} concentration which is regulated by abundance of metal ion homeostasis proteins and environmental factors like nutritional metal ion uptake. Intriguingly, a Zn^{2+} deficiency is found in many ASD patients.

In view of the data linking ProSAP2/Shank3, Zn^{2+} and synaptic transmission, one might postulate a simple model, where the transsynaptic signaling is determined (1) by the amount of ProSAP2/Shank3 at the postsynapse. While ProSAP2/Shank3 in a soluble pool contributes to the potential of the postsynapse to undergo activity-dependent changes, the PSD bound pool determines the actual transsynaptic signal. However, given a constant turnover between the two pools and a shift from the soluble to the PSD bound pool upon activity, the amount of ProSAP2/Shank3 in both pools contributes to the signaling output. Thus, the concentration (C) of ProSAP2/Shank3 (Pr2/SH3): $C_{Pr2/SH3}$ is comprised of the total amount of ProSAP2/Shank3 at the postsynapse in this model (Fig. 3), (2) by the releasable pool of Zn^{2+}. The releasable pool of Zn^{2+} might be determined for instance by the amount of MTs in the postsynapse or by the rate of influx of Zn^{2+} released from the presynapse. Unknowing the contribution of the putative sources for Zn^{2+}, the amount of Zn^{2+} available for ProSAP2/Shank3 proteins is defined as concentration (C) of Zn^{2+} (Zn): C_{Zn} in this model and determines the increase of ProSAP2/Shank3 after synaptic activity (Fig. 3). From this model, it can be concluded that for a glutamatergic synapse, to mediate pre- and postsynaptic activity-dependent changes, the presence of both, functional ProSAP2/Shank3 proteins and Zn^{2+} is important. On an ultra-structural level, Zn^{2+} supplementation or depletion causes alterations in PSD thickness and area *in vitro* (Grabrucker et al., 2011b). However, not all components of the PSD are linked to ProSAP2/Shank3. While much evidence indicates an interaction with GKAP, PSD95, and NMDAR, other PSD anchored receptors may have a weaker association with the ProSAP/Shank scaffold. Thus, the local Zn^{2+} concentration may affect the degree of higher order structure within the PSD in a way that certain "modules" will be selectively enriched. It is possible that this lack of synaptic "fine tuning" when Zn^{2+} levels are low during development results in a pathology on the synaptic level, but also on the circuit level. Thus, Zn^{2+} might provide a mechanism of gene/environment interaction via ProSAP2/Shank3 regulation and (maternal) Zn^{2+} deficiency might be a major risk factor for the development of ASDs.

In the coming years, studies of treatment to enhance glutamatergic synaptic signaling will reveal its potential in treatment of neuropsychiatric disorders such as ASDs. Therapies attempting to influence brain Zn^{2+}-levels by preventing release, blocking ion channels, supplementing Zn^{2+} and buffering Zn^{2+} concentration have become increasingly important in the treatment of neurological and neuropsychiatric diseases (Bitanihirwe and Cunningham, 2009). Especially in Phelan McDermid Syndrome, assessment of the Zn^{2+} status will be interesting and Zn^{2+} supplementation, influencing the ProSAP2/Shank3 levels provided by the intact copy of the gene may be a promising approach. However, Zn^{2+} is taken up actively into the CNS via the blood brain barrier and targeted Zn^{2+} delivery into the CNS may require novel tools to tackle this task (Grabrucker et al., 2011c,d).

AMG is supported by Baustein 3.2 (L.SBN.0083) and the DAAD.

REFERENCES

Adlard PA, Parncutt JM, Finkelstein DI, Bush AI. 2010. Cognitive loss in zinc transporter-3 knock-out mice: A phenocopy for the synaptic and memory deficits of Alzheimer's disease? J Neurosci 30(5):1631–1636.

Andrews GK. 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol 59(1):95–104.

Andrews GK. 2001. Cellular zinc sensors: MTF-1 regulation of gene expression. Biometals 14(3–4):223–237.

Arons MH, Thynne CJ, Grabrucker AM, Li D, Schoen M, Cheyne JE, Boeckers TM et al. 2012. Autism associated mutations in ProSAP2/Shank3 impair synaptic transmission and neurexin-neuroligin mediated transsynaptic signaling. J. Neurosci 32(43):14966–14978.

Assaf SY, Chung SH. 1984. Release of endogenous Zn2+ from brain tissue during activity. Nature 308(5961): 734–736.

Aviv T, Lin Z, Ben-Ari G, Smibert CA, Sicheri F. 2006. Sequence-specific recognition of RNA hairpins by the SAM domain of Vts1p. Nat Struct Mol Biol 13:168–176.

Baron MK, Böckers TM, Vaida B, Faham S, Gingery M, Sawaya MR, Salyer D, et al. 2006. An architectural framework that may lie at the core of the postsynaptic density. Science 311(5760):531–535.

Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, et al. 2010. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42(6):489–491.

Besser L, Chorin E, Sekler I, Silverman WF, Atkin S, Russell JT, Hershfinkel M. 2009. Synaptically released zinc triggers metabotropic signaling via a zinc-sensing receptor in the hippocampus. J Neurosci 29(9):2890–2901.

Bitanihirwe BK, Cunningham MG. 2009. Zinc: The brain's dark horse. Synapse 63(11):1029–1049.

Boeckers TM, Kreutz MR, Winter C, Zuschratter W, Smalla KH, Sanmarti-Vila L, Wex H, et al. 1999. Proline-rich synapse-associated protein-1/cortactin binding protein 1 (ProSAP1/CortBP1) is a PDZ-domain protein highly enriched in the postsynaptic density. J Neurosci 19(15):6506–6518.

Boeckers TM, Bockmann J, Kreutz MR, Gundelfinger ED. 2002. ProSAP/Shank proteins – A family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease. J Neurochem 81(5):903910.

Boeckers TM, Liedtke T, Spilker C, Dresbach T, Bockmann J, Kreutz MR, Gundelfinger ED. 2005. C-terminal synaptic targeting elements for postsynaptic density proteins ProSAP1/Shank2 and ProSAP2/Shank3. J Neurochem 92(3):519–524.

Bonaglia MC, Giorda R, Borgatti R, Felisari G, Gagliardi C, Selicorni A, Zuffardi O. 2001. Disruption of the ProSAP2 gene in a t(12;22)(q24.1;q13.3) is associated with the 22q13.3 deletion syndrome. Am J Hum Genet 69:261–268.

Bourgeron T. 2009. A synaptic trek to autism. Curr Opin Neurobiol 19 (2):231–234.

Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, Kajiwara Y, et al. 2011. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. Mol Autism 1:15.

Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD. 1999. Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. Proc Natl Acad Sci USA 96(4):1716–21.

Curtis LT, Patel K. 2008. Nutritional and environmental approaches to preventing and treating autism and attention deficit hyperactivity disorder (ADHD): A review. J Altern Complement Med 14(1):79–85.

De Rycker M, Price CM. 2004 Tankyrase polymerization is controlled by its sterile a motif and poly(ADP-ribose) polymerase domains. Mol Cell Biol 24:9802–9812.

Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, et al. 2007. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 39(1):25–27.

Erickson JC, Masters BA, Kelly EJ, Brinster RL, Palmiter RD. 1995. Expression of human metallothionein-III in transgenic mice. Neurochem Int 27 (1):35–41.

Erickson JC, Hollopeter G, Thomas SA, Froelick GJ, Palmiter RD. 1997. Disruption of the metallothionein-III gene in mice: Analysis of brain zinc, behavior, and neuron vulnerability to metals, aging, and seizures. J Neurosci 17(4):1271–1281.

Faber S, Zinn GM, Kern JC, Kingston HM. 2009. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers 14(3):171–180.

Fredericksen CJ, Koh JY, Bush AI. 2005. The neurobiology of zinc in health and disease. Nat Rev Neurosci 6: 449–462.

Gauthier J, Spiegelman D, Piton A, Lafrenière RG, Laurent S, St-Onge J, Lapointe L, et al. 2009. Novel de novo SHANK3 mutation in autistic patients. Am J Med Genet B Neuropsychiatr Genet 150B(3):421–424.

Golub MS, Keen CL, Gershwin ME, Hendrickx AG. 1995. Developmental zinc deficiency and behavior. J Nutr 125(8):2263–2271.

Grabrucker AM. 2012. Environmental factors in autism. Front Psych 3:118.

Grabrucker AM, Garner CC, Boeckers TM, Bondioli L, Ruozi B, Forni F, Vandelli MA, et al. 2011c. Development of novel Zn2+ loaded nanoparticles designed for cell-type targeted drug release in CNS neurons: In vitro evidences. Plos One 6(3):e17851.

Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, Nienhaus GU, et al. 2011b. Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. EMBO J. 30(3):569–581.

Grabrucker AM, Rowan M, Garner CC. 2011d. Brain-delivery of Zinc-ions as potential treatment for neurological diseases. Drug Deliv Lett 1(1):13–23.

Grabrucker AM, Schmeisser MJ, Schoen M, Boeckers TM. 2011a. Postsynaptic ProSAP/Shank scaffolds in the cross-hair of synaptopathies. Trends Cell Biol 21(10): 594–603.

Grabrucker S, Jannetti L, Eckert M, Gaub S, Chhabra R, Pfaender S, Mangus K, et al. 2013. Zinc deficiency dysregulates the synaptic ProSAP/Shank scaffold and might contribute to autism spectrum disorders. Brain [in press].

Gundelfinger ED, Boeckers TM, Baron MK, Bowie JU. 2006. A role for zinc in postsynaptic density asSAMbly and plasticity? Trends Biochem Sci 31:366–373.

Hall AC, Young BW, Bremner I. 1979. Intestinal metallothionein and the mutual antagonism between Cu^{2+} and zinc in the rat. J Inorg Biochem 11 (1):57–66.

Hagerman R, Hoem G, Hagerman P. 2010. Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. Mol Autism 1(1):12.

Hayashi MK, Tang C, Verpelli C, Narayanan R, Stearns MH, Xu RM, Li H, et al. 2009. The postsynaptic density

proteins Homer and Shank form a polymeric network structure. Cell 137(1):159–171.

Howell GA, Welch MG, Frederickson CJ. 1984. Stimulation-induced uptake and release of zinc in hippocampal slices. Nature 308(5961):736–738.

Huang YZ, Pan E, Xiong ZQ, McNamara JO. 2008. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546–558.

Huster D. 2010. Wilson disease. Best Pract Res Clin Gastroenterol 24(5):531–539.

Jackson KA, Valentine RA, Coneyworth LJ, Mathers JC, Ford D. 2008. Mechanisms of mammalian zinc-regulated gene expression. Biochem Soc Trans 36(Pt 6):1262–1266.

Jan HH, Chen IT, Tsai YY, Chang YC. 2002. Structural role of zinc ions bound to postsynaptic densities. J Neurochem 83:525.

Jen M, Yan AC. 2010. Syndromes associated with nutritional deficiency and excess. Clin Dermatol 28(6):669–685.

Koumura A, Kakefuda K, Honda A, Ito Y, Tsuruma K, Shimazawa M, Uchida Y, et al. 2009. Metallothionein-3 deficient mice exhibit abnormalities of psychological behaviors. Neurosci Lett 467(1):11–14.

Lakshmi Priya MD, Geetha A. 2011. Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 142(2): 148–158.

Levy D, Ronemus M, Yamrom B, Lee YH, Leotta A, Kendall J, et al. 2011. Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 70(5):886–897.

Li X, Zou H, Brown WT. 2012. Genes associated with autism spectrum disorder. Brain Res Bull 88(6):543–552.

Li Y, Hough CJ, Frederickson CJ, Sarvey JM. 2001. Induction of mossy fiber - CA3 long-term potentiation requires translocation of synaptically released Zn^{2+}. J Neurosci 21:8015–8025.

Lynch MA. 2004. Long-term potentiation and memory. Physiol Rev 84(1):87–136.

Manning MA, Cassidy SB, Clericuzio C, Cherry AM, Schwartz S, Hudgins L, Enns GM, et al. 2004. Terminal 22q deletion syndrome: A newly recognized cause of speech and language disability in the autism spectrum. Pediatrics 114:451–457.

Martel G, Hevi C, Friebely O, Baybutt T, Shumyatsky GP. 2010. Zinc transporter 3 is involved in learned fear and extinction, but not in innate fear. Learn Mem 17(11):582–590.

Meyer G, Varoqueaux F, Neeb A, Oschlies M, Brose N. 2004. The complexity of PDZ domain-mediated interactions at glutamatergic synapses: A case study on neuroligin. Neuropharmacology 47:724–733.

Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, et al. 2007. Contribution of SHANK3 mutations to autism spectrum disorder. Am J Hum Genet 81(6):1289–1297.

Naisbitt S, Kim E, Tu JC, Xiao B, Sala C, Valtschanoff J, Weinberg RJ, et al. 1999. Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. Neuron 23:569–582.

Nuttall JR, Oteiza PI. 2012. Zinc and the ERK kinases in the developing brain. Neurotox Res 21(1):128–141.

Oberstrass FC, Lee A, Stefl R, Janis M, Chanfreau G, Allain FH. 2006. Shape-specific recognition in the structure of the Vts1p SAM domain with RNA. Nat Struct Mol Biol 13:160–167.

O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Karakoc E, et al. 2011. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nat Genet 43(6):585–589.

Park JD, Liu Y, Klaassen CD. 2001. Protective effect of metallothionein against the toxicity of cadmium and other metals(1). Toxicology 163(2–3):93–100.

Pérez-Clausell J, Danscher G. 1985. Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. Brain Res 337(1):91–98.

Pfeiffer CC, Braverman ER. 1982. Zinc, the brain and behavior. Biol Psychiatry 17(4):513–532.

Phelan MC, Rogers RC, Saul RA, Stapleton GA, Sweet K, McDermid H, Shaw SR, et al. 2001. Research Review: 22q13 Deletion Syndrome. Am J Med Genet 101:91–99.

Phelan MC. 2008. Deletion 22q13.3 syndrome. Orphanet J Rare Dis 273(1):14.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, et al. 2010. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466(7304):368–72.

Qiao F, Song H, Kim CA, Sawaya MR, Hunter JB, Gingery M, Rebay I, et al. 2004. Derepression by depolymerization; structural insights into the regulation of Yan by Mae. Cell 118:163–173.

Qiao F, Bowie JU. 2005. The many faces of SAM. Sci STKE 2005, re7.

Qiao F, Harada B, Song H, Whitelegge J, Courey AJ, Bowie JU. 2006. Mae inhibits Pointed-P2 transcriptional activity by blocking its MAPK docking site. EMBO J 25:70–79.

Roussignol G, Ango F, Romorini S, Tu JC, Sala C, Worley PF, Bockaert J, et al. 2005. Shank expression is sufficient to induce functional dendritic spine synapses in aspiny neurons. J Neurosci 25:3560–3570.

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, et al. 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485 (7397):237–241.

Sandstead HH, Strobel DA, Logan GM, Marks EO, Jacob RA. 1978. Zinc deficiency in pregnant rhesus monkeys: Effects on behavior of infants. Am J Clin Nutr 31(5):844–849.

Sato D, Lionel AC, Leblond CS, Prasad A, Pinto D, Walker S, O'Connor I, et al. 2012. SHANK1 deletions in males

with autism spectrum disorder. Am J Hum Genet 90(5):879–887.

Schmeisser MJ, Ey E, Wegener S, Bockmann J, Stempel AV, Kuebler A, Janssen AL, et al. 2012. Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature 486(7402):256–260.

Serajee FJ, Nabi R, Zhong H, Huq M. 2004. Polymorphisms in xenobiotic metabolism genes and autism. J Child Neurol 19(6):413–417.

Shankar AH, Prasad AS. 1998. Zinc and immune function: The biological basis of altered resistance to infection. Am J Clin Nutr 68:447–463.

Sindreu CB, Varoqui H, Erickson JD, Pérez-Clausell J. 2003. Boutons containing vesicular zinc define a subpopulation of synapses with low AMPAR content in rat hippocampus. Cereb Cortex 13(8):823–829.

Sindreu C, Palmiter RD, Storm DR. 2011. Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. Proc Natl Acad Sci USA 108(8):3366–3370.

Sindreu C, Storm DR. 2011. Modulation of neuronal signal transduction and memory formation by synaptic zinc. Front Behav Neurosci 5:68.

Song H, Nie M, Qiao F, Bowie JU, Courey AJ. 2005. Antagonistic regulation of Yan nuclear export by Mae and Crm1 may increase the stringency of the Ras response. Genes Dev 19:1767–1772.

Trabacca A, Losito L, De Rinaldis M, Gennaro L. 2011. Congenital hypotonia in a child with a de novo 22q13 monosomy and 2pter duplication: A clinical and molecular genetic study. J Child Neurol 26(2):235–238.

Tuchman R, Cuccaro M. 2011. Epilepsy and autism: Neurodevelopmental perspective. Curr Neurol Neurosci Rep 11(4):428–434.

Verpelli C, Dvoretskova E, Vicidomini C, Rossi F, Chiappalone M, Schoen M, Di Stefano B, et al. 2011. Importance of shank3 in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. J Biol Chem 286(40):34839–34850.

Vincent SR, Semba K. 1989. A heavy metal marker of the developing striatal mosaic. Brain Res Dev Brain Res 45(1):155–159.

Walsh WJ, Usman A, Tarpey J. 2001. Disordered metal metabolism in a large autism population, Proceedings of the Amer Psych Assn; New Research: Abstract NR109, New Orleans.

Walsh WJ, Usman A, Tarpey J, Kelly T. 2002. Metallothionein and Autism, 2nd ed. Naperville, IL: Pfeiffer Treatment Center.

Wang X, Zhou B. 2010. Dietary zinc absorption: A play of Zips and ZnTs in the gut. IUBMB Life 62(3):176–82.

Wilson HL, Wong ACC, Shaw SR, Tse W-Y, Stapleton GA, Phelan MC, Hu S, et al. 2003. Molecular characterization of the 22q13 deletion syndrome supports the role of haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. J Med Gen 40:575–584.

Wimmer U, Wang Y, Georgiev O, Schaffner W. 2005. Two major branches of anti-cadmium defense in the mouse: MTF-1/metallothioneins and glutathione. Nucleic Acids Res 33 (18):5715–5727.

Wong AC, Bell CJ, Dumanski JP, Budarf ML, McDermid, HE. 1995. Molecular characterization of a microdeletion at 22q13.3. Am J Hum Genet 57:A130.

Yasuda H, Yoshida K, Yasuda Y, Tsutsui T. 2011. Infantile zinc deficiency: Association with autism spectrum disorders. Sci Rep 1:129.