

TSPAN7

A new player in excitatory synapse maturation and function

Silvia Bassani and Maria Passafaro*

CNR Institute of Neuroscience; Department of Medical Pharmacology; University of Milan; Milan, Italy

Keywords: intellectual disability, AMPAR trafficking, synapse function/plasticity, tetraspanins, TSPAN7, integrins, PICK1

Abbreviations: AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDAR, *N*-methyl-D-aspartate receptor; PI4K, phosphatidylinositol 4-kinase; PKC, protein kinase C; PICK1, protein interacting with C kinase 1; TEMs, tetraspanin enriched microdomains; PSD, post synaptic density

Submitted: 05/18/12

Revised: 05/21/12

Accepted: 05/21/12

<http://dx.doi.org/10.4161/bioa.20829>

*Correspondence to: Maria Passafaro;
Email: diantonio@wustl.edu

Commentary to: Bassani S, Cingolani LA, Valnegri P, Folci A, Zapata J, Gianfelice A, et al. The X-linked intellectual disability protein TSPAN7 regulates excitatory synapse development and AMPAR trafficking. *Neuron* 2012; 73:1143–58; PMID:22445342; <http://dx.doi.org/10.1016/j.neuron.2012.01.021>

Tetraspanins regulate the signaling, trafficking and biosynthetic processing of associated proteins, and may link the extracellular domain of α -chain integrins with intracellular signaling molecules, including PI4K and PKC, both of which regulate cytoskeletal architecture. We showed that TSPAN7, a member of tetraspanin-family, promotes filopodia and dendritic spine formation in cultured hippocampal neurons, and is required for spine stability and normal synaptic transmission. TSPAN7 directly interacts with the PDZ domain of protein interacting with C kinase 1 (PICK1), and associates with AMPAR subunit GluA2 and β 1-integrin. TSPAN7 regulates PICK1 and GluA2/3 association, and AMPA receptor trafficking. These findings identify TSPAN7 as a key player in the morphological and functional maturation of glutamatergic synapses.

Tetraspanins are evolutionarily-conserved membrane proteins that associate dynamically with numerous partner proteins in tetraspanin enriched microdomains (TEMs) and regulate cell morphology, motility, and signaling.¹ Many of the 33 mammalian tetraspanins are expressed in the nervous system, playing a role in neurite outgrowth, myelination and glia cell number control.² Among them TSPAN7, encoded by the *TM4SF2* gene, is directly associated with cognitive defects in humans. *TM4SF2* mutations, including *TM4SF2* inactivation by an X:2 balanced translocation, a premature stop codon TGA (gly218-to-ter),³ and a 2-bp deletion (564delGT) resulting in a premature stop codon at position 192⁴ are directly

associated with non-syndromic intellectual disability. The gly218-to-ter nonsense mutation and the 2-bp deletion predict a truncated protein lacking the fourth transmembrane domain and cytoplasmic C-terminal tail.

In our recent paper⁵ we have identified TSPAN7 as a new player in synaptic maturation and function, showing that it localizes at excitatory synapses in cultured embryonic rat neurons.

In particular, we found that TSPAN7 overexpression promotes the formation of filopodia and dendritic spines, while TSPAN7 knockdown causes a reduction in spine head size, and increases spine motility and turnover—consistent with the results of an *in vivo* study showing that small filopodial-like spines are more dynamic and undergo more rapid remodelling than larger spines.⁶ These morphological abnormalities are similar to those reported in some mentally retarded patients⁷ and also in various animal models of mental disorders^{7,8} including oligophrenin-1-, *Il-1RAPL1*-, and *PAK3*- knockout mice;⁹ similar morphological abnormalities have also been observed in dissociated neuronal cultures using specific siRNAs to knock-down the transcripts of the same proteins.⁹

It is noteworthy that changes in spine morphology are closely linked to changes in the strength of synaptic connections.¹⁰ Thus, mushroom spines have larger, more complex postsynaptic densities (PSDs), with higher glutamate receptor densities, than smaller spines;¹¹ furthermore spine size correlates with PSD size.¹² The fact that TSPAN7 knockdown affects spine morphology suggests it is causing defects in PSDs and reducing the stability of postsynaptic structures. Consistent with

this, TSPAN7 knockdown reduced the expression level of postsynaptic markers, in particular of AMPARs, and such decreases were accompanied by a reduction in the number of functional synaptic AMPARs as shown by a decrease in mEPSCs amplitude and frequency. The measure of PPR is not altered upon TSPAN7 knockdown, consistent with an increase in the number of silent synapses, i.e., lacking AMPAR, in silenced neurons. By contrast NMDAR currents were not affected.

It is important to examine how TSPAN7 may affect PSD composition and AMPAR currents. As tetraspanins associate with several proteins and regulate their trafficking and signaling,¹ the function of these molecules has to be considered in the light of their specific interacting/binding proteins. It has previously been reported that TSPAN7 associates with phosphatidylinositol 4-kinase (PI4K) type II activity.¹³ In this paper, we identified other TSPAN7 associated proteins: PICK1, that is a direct interactor and GluA2 AMPAR subunit and β 1-integrin that are associated to TSPAN7 (Fig. 1). We showed that the C-terminal tail of TSPAN7 interacts with the PICK1

PDZ domain. We also found that TSPAN7 associates with GluA2/3 and β 1-integrin, and that following TSPAN7 knockdown, PICK1 associated more tightly with AMPAR, while β 1-integrin no longer did so. These findings suggest that these proteins form a single macromolecular complex, whose composition depends on TSPAN7.

TSPAN7 could, therefore, regulate signaling pathways important for synaptic transmission and plasticity via its association with β 1-integrin, PICK1 and AMPA. PICK1 is a well-established regulator of AMPAR trafficking and of the number of AMPA receptors at synapses.¹⁴ It has been involved in AMPAR-regulated trafficking in long-term depression (LTD) in hippocampal neurons^{15,16} and the cerebellum.^{17,18} Changes in AMPAR trafficking are crucial to synaptic plasticity and therefore to learning and memory. In fact, in various synapse types, LTD involves endocytosis of AMPARs from the plasma membrane, whereas long-term potentiation (LTP) involves insertion of additional AMPARs to the surface.^{19,20} We found that GluA2 underwent increased internalization in response to TSPAN7

knockdown, with reduction in surface GluA2, that was accompanied by decreased mEPSCs; by contrast endocytosis block with dynasore restored GluA2 surface levels, pointing out AMPAR trafficking as the mechanism involved. The modulation of PICK1 and TSPAN7 expression independently leads to opposite effects on GluR2 distribution, with PICK1 overexpression promoting—and PICK1 silencing reducing—GluA2 internalization,^{21,22} while the TSPAN7 expression level positively correlates with surface localization of GluA2 containing AMPAR. Rebalancing the relative expression of the two proteins by double knockdown or double overexpression experiments reinstate GluA2 distribution to control levels. Interestingly TSPAN7 deleted for its C-terminus and thus lacking the amino acids responsible for PICK1 binding, has no effect on GluA2 trafficking. These data are consistent with a model in which TSPAN7 competes with AMPAR for PICK1 binding and identify TSPAN7 as a new modulator of AMPAR trafficking through PICK1.

TSPAN7's direct binding to PICK1 suggests a ready explanation for the defects in AMPAR trafficking observed upon TSPAN silencing, but does not explain the effects of TSPAN7 on filopodia and dendritic spine morphology. A possible explanation is via the regulation of actin filaments. The initiation and elongation of filopodia as well as spine formation are known to depend on the precisely regulated polymerization and cross-linking of actin filaments.²³ Although PICK1 is also involved in actin polymerization,²⁴ we showed that PICK1 and TSPAN7 regulate spine morphology independently. A possibility is that TSPAN7 influences actin filaments via an association with PI4K¹³ and/or via its association with β 1-integrin—as shown in this and other studies.²⁵ Both PI4K and β 1-integrin are known regulators of actin dynamics.^{26,27} By linking these two proteins, TSPAN7 could recruit them to a complex on the plasma membrane that coordinates actin dynamics with the assembly or organization of postsynaptic AMPA receptors.

We investigated the presence of β 1-integrin in the TSPAN7 complex since tetraspanins are known to interact with

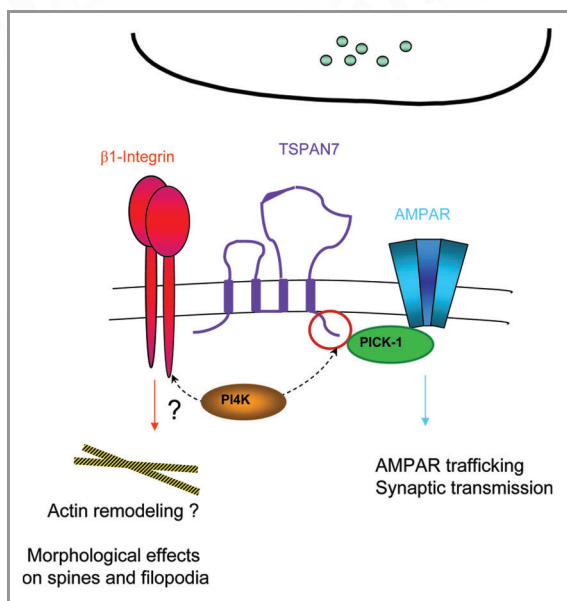


Figure 1. The cartoon shows a synaptic complex composed by TSPAN7 and the proteins: PICK1, AMPAR, integrins and PI4K. In particular, TSPAN7 associates with AMPAR GluA2 subunit, β 1-integrin, PI4K and, through its C-terminal tail, directly binds PICK1 PDZ domain. Through PICK1, TSPAN7 modulates AMPAR trafficking thus regulating synaptic function. A distinct mechanism, likely involving actin remodelling pathways, accounts for TSPAN7 regulation of filopodia density and dendritic spine morphology. We speculate that this involves the β 1-integrin and PI4K partners.

integrins (especially those containing the beta1 chain)²⁵ and β 1-integrin is involved in LTP,²⁸ suggesting involvement in learning and memory. In fact, mice with postnatal deletion of β 1-integrin are impaired in working memory.²⁸ We found that β 1-integrin is part of the TSPAN7-AMPA complex.

Other studies have shown that regulatory interactions between integrins and ion channels are common and often involve formation of a multi-protein complex² and that glutamate receptors are targets for integrins.² In dissociated neurons and cultured hippocampal slices, AMPA treatment has been shown to increase the surface

expression of β 1- and α 5-integrin.²⁹ Our data indicate that TSPAN7 serves as a functional connector between integrins and AMPARs with implications for synaptic transmission and plasticity particularly since AMPARs mediate the majority of fast excitatory synaptic transmission in the brain, and changes in AMPAR number or function, can result in alterations in synaptic strength¹⁹ (Fig. 1).

The changes we have observed in synapse morphology and physiology resulting from TSPAN7 silencing point to TSPAN7 having a critical role in synapse function, consistent with the intellectual disability found in some

patients with TSPAN7 mutations. Our findings allow us to speculate that TSPAN7-related intellectual disability may occur because TSPAN7 loss results in alteration of dendritic filopodia and spine morphology and synapse physiology, likely mediated by reduced stabilization of AMPAR at dendritic spines and also by lack of binding of β 1-integrin to the AMPAR-PICK1 complex.

Acknowledgments

We thank the Telethon Fondation (S01014TELU), Fondazione Cariplo (2008-2318) and Fondazione Mariani for their support.

References

- Hemler ME. Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol* 2005; 6:801-11; PMID:16314869; <http://dx.doi.org/10.1038/nrm1736>
- Bassani S, Cingolani LA. Tetraspanins: Interactions and interplay with integrins. *Int J Biochem Cell Biol* 2012; 44:703-8; PMID:22326999; <http://dx.doi.org/10.1016/j.biocel.2012.01.020>
- Zemni R, Bienvenu T, Vinet MC, Sefiani A, Carrié A, Billuart P, et al. A new gene involved in X-linked mental retardation identified by analysis of an X;2 balanced translocation. *Nat Genet* 2000; 24:167-70; PMID:10655063; <http://dx.doi.org/10.1038/72829>
- Abidi FE, Holinski-Feder E, Rittinger O, Kooy F, Lubs HA, Stevenson RE, et al. A novel 2 bp deletion in the TM4SF2 gene is associated with MRX58. *J Med Genet* 2002; 39:430-3; PMID:12070254; <http://dx.doi.org/10.1136/jmg.39.6.430>
- Bassani S, Cingolani LA, Valnegri P, Folci A, Zapata J, Gianflice A, et al. The X-linked intellectual disability protein TSPAN7 regulates excitatory synapse development and AMPAR trafficking. *Neuron* 2012; 73:1143-58; PMID:22445342; <http://dx.doi.org/10.1016/j.neuron.2012.01.021>
- Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, et al. Transient and persistent dendritic spines in the neocortex in vivo. *Neuron* 2005; 45:279-91; PMID:15664179; <http://dx.doi.org/10.1016/j.neuron.2005.01.003>
- Purpura DP. Dendritic spine "dysgenesis" and mental retardation. *Science* 1974; 186:1126-8; PMID:4469701; <http://dx.doi.org/10.1126/science.186.4169.1126>
- Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am J Med Genet* 2001; 98:161-7; PMID:11223852; [http://dx.doi.org/10.1002/1096-8628\(20010115\)98:2<161::AID-AJMG1025>3.0.CO;2-B](http://dx.doi.org/10.1002/1096-8628(20010115)98:2<161::AID-AJMG1025>3.0.CO;2-B)
- Boda B, Mas C, Muller D. Activity-dependent regulation of genes implicated in X-linked non-specific mental retardation. *Neuroscience* 2002; 114:13-7; PMID:12207950; [http://dx.doi.org/10.1016/S0306-4522\(02\)00218-X](http://dx.doi.org/10.1016/S0306-4522(02)00218-X)
- Yuste R, Bonhoeffer T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu Rev Neurosci* 2001; 24:1071-89; PMID:11520928; <http://dx.doi.org/10.1146/annurev.neuro.24.1.1071>
- Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J. Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci* 2010; 33:121-9; PMID:20138375; <http://dx.doi.org/10.1016/j.tins.2010.01.001>
- Bourne HR, Nicoll R. Molecular machines integrate coincident synaptic signals. *Cell* 1993; 72(Suppl):65-75; PMID:8094038; [http://dx.doi.org/10.1016/S0092-8674\(05\)80029-7](http://dx.doi.org/10.1016/S0092-8674(05)80029-7)
- Yauch RL, Hemler ME. Specific interactions among transmembrane 4 superfamily (TM4SF) proteins and phosphoinositide 4-kinase. *Biochem J* 2000; 351:629-37; PMID:11042117; <http://dx.doi.org/10.1042/0264-6021:3510629>
- Hanley JG. PICK1: a multi-talented modulator of AMPA receptor trafficking. *Pharmacol Ther* 2008; 118:152-60; PMID:18353440; <http://dx.doi.org/10.1016/j.pharmthera.2008.02.002>
- Kim CH, Lisman JE. A labile component of AMPA receptor-mediated synaptic transmission is dependent on microtubule motors, actin, and N-ethylmaleimide-sensitive factor. *J Neurosci* 2001; 21:4188-94; PMID:11404404
- Hanley JG. Molecular mechanisms for regulation of AMPAR trafficking by PICK1. *Biochem Soc Trans* 2006; 34:931-5; PMID:17052230; <http://dx.doi.org/10.1042/BST0340931>
- Xia J, Chung HJ, Wihler C, Haganir RL, Linden DJ. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron* 2000; 28:499-510; PMID:11444359; [http://dx.doi.org/10.1016/S0896-6273\(00\)00128-8](http://dx.doi.org/10.1016/S0896-6273(00)00128-8)
- Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S, et al. Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. *Neuron* 2006; 49:845-60; PMID:16543133; <http://dx.doi.org/10.1016/j.neuron.2006.02.025>
- Passafaro M, Pièch V, Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat Neurosci* 2001; 4:917-26; PMID:11528423; <http://dx.doi.org/10.1038/nn0901-917>
- Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 2002; 25:103-26; PMID:12052905; <http://dx.doi.org/10.1146/annurev.neuro.25.112701.142758>
- Jin W, Ge WP, Xu J, Cao M, Peng L, Yung W, et al. Lipid binding regulates synaptic targeting of PICK1, AMPA receptor trafficking, and synaptic plasticity. *J Neurosci* 2006; 26:2380-90; PMID:16510715; <http://dx.doi.org/10.1523/JNEUROSCI.3503-05.2006>
- Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB. PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. *J Neurosci* 2001; 21:5417-28; PMID:11466413
- Cingolani LA, Goda Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nat Rev Neurosci* 2008; 9:344-56; PMID:18425089; <http://dx.doi.org/10.1038/nrn2373>
- Rocca DL, Martin S, Jenkins EL, Hanley JG. Inhibition of Arp2/3-mediated actin polymerization by PICK1 regulates neuronal morphology and AMPA receptor endocytosis. *Nat Cell Biol* 2008; 10:259-71; PMID:18297063; <http://dx.doi.org/10.1038/ncb1688>
- Berditchevski F. Complexes of tetraspanins with integrins: more than meets the eye. *J Cell Sci* 2001; 114:4143-51; PMID:11739647
- Clegg DO, Wingerd KL, Hikita ST, Tolhurst EC. Integrins in the development, function and dysfunction of the nervous system. *Front Biosci* 2003; 8:d723-50; PMID:12700040; <http://dx.doi.org/10.2741/1020>
- Hilpelä P, Vartiainen MK, Lappalainen P. Regulation of the actin cytoskeleton by PI(4,5)P2 and PI(3,4,5)P3. *Curr Top Microbiol Immunol* 2004; 282:117-63; PMID:14594216; http://dx.doi.org/10.1007/978-3-642-18805-3_5
- Chan CS, Weeber EJ, Zong L, Fuchs E, Sweatt JD, Davis RL. Beta 1-integrins are required for hippocampal AMPA receptor-dependent synaptic transmission, synaptic plasticity, and working memory. *J Neurosci* 2006; 26:223-32; PMID:16399691; <http://dx.doi.org/10.1523/JNEUROSCI.4110-05.2006>
- Lin CY, Lynch G, Gall CM. AMPA receptor stimulation increases alpha5beta1 integrin surface expression, adhesive function and signaling. *J Neurochem* 2005; 94:531-46; PMID:16000124; <http://dx.doi.org/10.1111/j.1471-4159.2005.03203.x>