COMMENTARY

Why we need more synaptogenic cell-adhesion proteins

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The mammalian brain is arguably the most complex organ that evolution has brought forward. In the human brain, for example, about 10^{11} neurons are connected via some 10^{15} synapses to form multiple specifically connected neural networks, the interplay of which controls all body functions. Synapses, the key signaling units in the brain, represent yet another level of substantial complexity; they are intricate molecular machines composed of thousands of different proteins. The questions as to how the specificity of synaptic connections in the face of huge neuron and synapse numbers is achieved during brain development, and how the molecular composition of synapses is regulated, have been in the focus of neuroscientists for decades. In PNAS, Yim et al. (1) report that different cell-adhesion proteins of the Slitrk (Slit- and Trk-like) family interact with different cell-surface–exposed protein tyrosine phosphatases (PTPs), and thereby allow neurons to recognize each other during synapse formation and establish different synapse types.

Synapses operate by chemical signaling. The sending part of the synapse, the presynaptic bouton, is specialized for the regulated exocytosis of neurotransmitters, which is triggered by membrane depolarization and Ca^{2+} influx. Released neurotransmitters bind to receptor proteins on the surface of the receiving part of the synapse, the postsynaptic density, and thereby elicit a membrane potential change in the receiving cell. Most synapses in the mammalian forebrain use the neurotransmitter glutamate, which binds to receptors that act as ligand-gated cation channels and cause membrane depolarization and excitation of the receiving cell. Glutamatergic excitatory synaptic transmission in the forebrain is counterbalanced by the activity of inhibitory synapses. Most of these synapses use the neurotransmitter GABA, whose $GABA_A$ receptors are ligand-gated chloride channels that hyperpolarize and thus inhibit the receiving cell. The excitation-inhibition balance in the brain is maintained within narrow boundaries. Correspondingly, persistent alterations in neuronal and network activity trigger homeostatic changes in the function and number of excitatory and inhibitory synapses to guarantee an appropriate excitation-inhibition balance (2), and pathological changes of this balance cause brain diseases, such as epilepsy (3) or autism (4).

Synapses are thought to form in a two-step process, where an initial cell-type–specific contact between two neurons is formed that subsequently matures into a fully functional synapse. Both, the cell-type–specific formation of a nascent synaptic contact and its subsequent maturation, which involves the recruitment of the presynaptic and postsynaptic protein machineries required for proper synaptic signaling, are mediated by synaptic adhesion proteins (5). Because the corresponding postsynaptic receptors and scaffolding proteins are strikingly different in glutamatergic and GABAergic synapses (Fig. 1), the protein-recruitment processes involved in the formation and maturation of the two synapse types must be fundamentally different. A substantial number of different synaptic cell-adhesion systems have been shown to control the formation
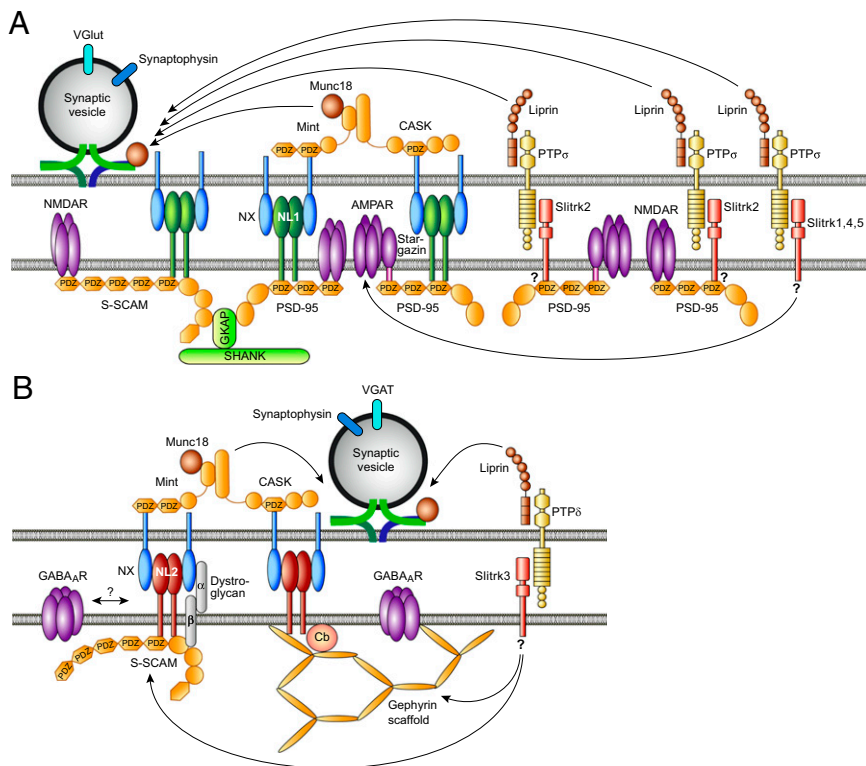

Fig. 1. Comparison of the neurexin-neuroligin and Slitrk-PTP adhesion systems at excitatory (A) and inhibitory (B) synapses. See text for details. AMPAR, AMPA receptor; CASK, calcium/calmodulin-dependent serine protein kinase; Cb, Collybistin; GABAAR, GABAA receptor; GKAP, guanylate kinase associated protein; MINT, Munc18 interacting protein; NL, Neuroligin; NMDAR, NMDA receptor; NX, Neurexin; PDZ, PSD-95/disk-large/zona-occludens-1 domain; PSD-95, postsynaptic density protein of 95 kDa; PTP, protein tyrosine phosphatase; SHANK, SH3 and ankyrin repeat containing protein; S-SCAM, synaptic scaffolding molecule; Slitrk, Slit- and Trk-like; VGAT, vesicular GABA/glycine transporter; VGlut, vesicular glutamate transporter.

of glutamatergic excitatory synapses, and most of them operate by binding PDZ-domain–containing scaffold proteins such as PSD-95, which then—directly or indirectly—recruit glutamate receptors to the maturing synapse (6). In contrast, GABAergic post-synapses contain very few PDZ-domain proteins and instead require the scaffold protein gephyrin and, in many cases, its recruitment factor collybistin to accumulate $GABA_A$ receptors. As regards the corresponding cell-adhesion proteins involved, only the α/β-dystroglycan complex and two members of the neuroligin family, neuroligin-2 and neuroligin-4, have been shown to operate in the formation and maturation of inhibitory postsynaptic signaling complexes (7).

The α/β-dystroglycan complex binds pre-synaptic adhesion proteins of the neurexin family and the intracellular scaffold protein S-SCAM, but how this leads to $GABA_A$ receptor recruitment to synapses is unknown. Neuroligin-2 and neuroligin-4 also bind pre-synaptic neurexins, but in contrast to the α/β-dystroglycan complex, their intracellular C termini interact directly with gephyrin and bind and activate collybistin, which leads to the postsynaptic recruitment of the two scaffold proteins and subsequent post-synaptic assembly of $GABA_A$ receptors (7). Strikingly, however, only a subset of inhibitory synapses is dependent upon the presence of neuroligin-2, neuroligin-4, or collybistin, whereas gephyrin is obligatory for most inhibitory synapses (7). Thus, to explain the omnipresence and diversity of inhibitory synapses in the brain, there is a dire need for alternative synaptogenic adhesion proteins that can operate instead of neuroligin-2 or neuroligin-4 and their presynaptic interaction partners and that can trigger $GABA_A$ receptor recruitment in a partly collybistin-independent manner.

It is this need that the PNAS article by Yim et al. (1) addresses. Extending a previous study by others (8), the authors show that different members of the Slitrk family of postsynaptic cell-adhesion proteins interact with different presynaptic receptor PTPs to trigger the formation and maturation of excitatory glutamatergic or inhibitory

GABAergic synapses (Fig. 1). Specifically, Slitrk3 interacts with PTPδ to trigger the genesis of inhibitory GABAergic synapses, whereas Slitrk1, Slitrk2, Slitrk4, and Slitrk5 act at excitatory glutamatergic synapses by interacting with PTPσ. The action of Slitrk3

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at inhibitory synapses is phenomenologically related to that of neuroligin-2 and neuroligin-4 (7), whereas the effects of the other Slitrk family members are reminiscent of most synaptogenic proteins at excitatory synapses, including neuroligin-1 (6).

The molecular mechanisms by which PTPδ, PTPσ, and the different Slitrks trigger synapse formation and maturation are probably different from the ones seen with well-established presynaptically or postsyn-aptically localized synaptogenic adhesion proteins, such as neurexins or neuroligins. Whereas neurexins seem to trigger presyn-apse differentiation by binding to calcium/calmodulin-dependent serine protein kinase (9), PTPδ and PTPσ are likely to operate by directly interacting with Liprins (10). On the postsynaptic side, neuroligin-1 and most other postsynaptic adhesion proteins of ex-citatory synapses operate by interaction with

PDZ-domain–containing scaffold proteins (6), and neuroligin-2 and neuroligin-4 inter-act with collybistin and gephyrin to initiate the differentiation of inhibitory postsynapses (7). In contrast, none of the Slitrks except Slitrk2 has a potential PDZ-domain binding motif that would allow interactions with the most prevalent scaffold proteins of gluta-matergic synapses, and Slitrk3, which may be one of the enigmatic cronies of neuroligin-2 and neuroligin-4 in inhibitory synapse formation, lacks recognizable binding sites for collybistin or gephyrin.

The considerations above lead to the fascinating conclusion that Slitrks might exert their synaptogenic effects by unique mecha-nisms, the elucidation of which represents the most pressing open task left by Yim et al.'s report (1). The fascination of the re-cent findings and the resulting conclusions are particularly evident with regard to the role of Slitrk3 at inhibitory synapses. The for-mation and maturation of many GABAergic synapses in the mammalian forebrain appear to be independent of neuroligin-2, neuro-ligin-4, or collybistin (7). Therefore, homologs or analogs of neuroligin-2, neuroligin-4, and collybistin must exist that operate by similar mechanisms to recruit gephyrin and $GABA_A$ receptors to maturing inhibitory synapses. Beyond neuroligin-3, which is present at a subset of inhibitory synapses but does not bind collybistin (7), Slitrk3 is a very inter-esting candidate substitute for neuroligin-2 and neuroligin-4. It will be interesting to see if the synaptogenic effects of Slitrk3 in-volve the recruitment of the well-established scaffold proteins of inhibitory synapses or whether it operates by a different mechanism.

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