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JB Review

Orchestrating the synaptic network by tyrosine phosphorylation signalling

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The establishment of a functional brain requires coordinated and stereotyped formation of synapses between neurons. For this, trans-synaptic molecular cues (synaptic organizers) are exchanged between a neuron and its target to organize appropriate synapses. The understanding of signalling mechanisms by which such synaptic organizers lead to synapse formation is just being elucidated. However, recent studies revealed that some of these cues act through receptor protein tyrosine kinases (RPTKs) or phosphatases (RPTPs). Synaptogenic RPTKs and RPTPs pattern synaptic network through affecting local protein–protein binding dynamics, changing the phosphorylation state of signalling cascades, or promoting gene expression. Each RPTK or RPTP has distinct roles in synapse formation, serving at different synapses or showing differential synaptogenic effects. Thus, tyrosine phosphorylation signalling plays critical roles in building the orchestrated synaptic circuitry in the brain.

Keywords: receptor tyrosine kinases/receptor tyrosine phosphatases/signalling/synapse formation/synaptic organizers.

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate; AMPAR, AMPA-sensitive glutamate receptor; BDNF, brain-derived neurotrophic factor; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase; CREB, cAMP responsive element binding protein; CNS, central nervous system; DIV, days in vitro; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GABA, gamma-aminobutyric acid; GAD, guanine-exchange factor; GEF, guanine-exchange factor; KO, knock-out; LAR, leukocyte antigen related; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; NGF, nerve growth factor; NGL, netrin-G ligand; NMDA, N-methyl-D-aspartate; NMDAR, NMDA-sensitive glutamate receptor; PI-3K, phosphoinositol-3 kinase; PLCγ, phospholipase C gamma; PSD, postsynaptic density; PTP, protein tyrosine phosphatase; PV+, parvalbumin positive; RPTK, receptor protein tyrosine kinase; RPTP, receptor protein tyrosine phosphatase; SV, synaptic vesicle; VGCC, voltage-gated calcium channel.

The central nervous system (CNS) is composed of an astonishing number of cells, which encode processing of experience and behaviour through an interconnected network of neurons joined by synapses. Neurons must establish spatially and functionally appropriate connections with microscale precision, which requires coordination of extracellular events and intracellular processes (*1*). A plethora of molecules could promote aspects of synaptic development, including fibroblast growth factors (FGFs), neuroligins, neuregulins, ephrins, netrin-G ligands, leucine rich repeat transmembrane proteins (LRRTMs), SynCAMs, Wnts and neurotrophic factors (*2*, *3*). These molecules, which we call synaptic organizers, are shown to communicate between neurons to organize proper synapses. What are the signalling mechanisms underlying synapse formation mediated by these synaptic organizers? Notably, a subset of the synapse organizers binds to a receptor protein tyrosine kinase (RPTK) or receptor protein tyrosine phosphatase (RPTP). RPTKs and RPTPs could pose as important and unique players in transducing signals for synapse formation, because they can transform extracellular cues into both microlocal and broadscale effects. Locally, RPTKs and RPTPs can recruit synaptic components at the developing synapse through protein–protein interactions and localized changes in the phosphorylation state. Such local control may provide each synapse, which may be found within micrometers of each other, with distinct properties. Globally, RPTKs and RPTPs lead to changes in gene expression and thus promote synapse maturation even at distant synapses. The intracellular signalling pathways downstream of the receptors in the context of synapse formation are also beginning to be revealed. Here, we review the specific roles of synaptogenic RPTKs [FGF receptors (FGFRs), ErbB4, TrkA and Ephs] and RPTPs [leukocyte-antigen related (LAR) and protein tyrosine phosphatase σ (PTPσ)] and their signalling pathways in synapse formation, and discuss emerging principles in coordinating synapse formation.

Featured Article

Synapse formation

Synapses transmit information between neurons by encoding electrical activity as chemical signals. Neurotransmitter molecules are released into the synaptic cleft from axon terminals of presynaptic cells in response to electrical action potentials. Subsequent binding of neurotransmitters to postsynaptic receptors causes ion channel opening, which generates a postsynaptic electrical potential. Synapses where a neurotransmitter (glutamate) causes an increased probability of postsynaptic action potential firing are excitatory synapses, and conversely, synapses where a neurotransmitter [in general, gamma-aminobutyric acid (GABA)] causes a decreased probability of firing an action potential are inhibitory. A typical neuron (excitatory or inhibitory) releases only one type of neurotransmitter but receives many different inputs. Thus, localization of neurotransmitter receptors along a dendrite must be coordinated to the specific synaptic inputs. Assembly of proper presynaptic and postsynaptic structures in apposition to one another is an endpoint of synapse formation, allowing for efficient information transfer and establishment of a balanced synaptic network for the optimal functioning of the brain.

Synaptogenesis proceeds in three overall steps: first, initial contact is made between the axon and dendrite; second, synapse structures differentiate; and lastly, the synapse maturates (Fig. 1). In the rodent cortex and hippocampus, synaptogenesis peaks during the first 3 to 4 weeks postnatally, with the highest rate of synapse formation around postnatal day 7 to 14 (P7–P14) (*4–6*). Neurons from embryos or newborn pups can be dissociated and cultured; *in vitro* synapse formation parallels that *in vivo*. Synapses begin to form at approximately 4 days *in vitro* (DIV) from the time of plating and a peak in rate of formation is seen around 7–14DIV, after which synapses may continue to form but at a slower rate. By 21DIV, neurons are considered to form mature networks (*7–9*). During synapse formation, various components of pre- and postsynaptic machineries are assembled at nascent synapses (Fig. 1): active zones form, and synaptic vesicles and Ca^{2+} channels accumulate at presynaptic terminals; at postsynaptic sites, neurotransmitter receptors cluster, and postsynaptic densities (PSDs) and spines (for excitatory synapses) form (*10*). The coordination of synapse assembly requires the binding of pre- and postsynaptic organizers to the contacting axons or dendrites followed by schematic waves of signalling, including tyrosine phosphorylation signalling.

RPTKs and RPTPs

One way to conceptualize RPTK and RPTP is as nodes of signalling—a ligand binds to the extracellular

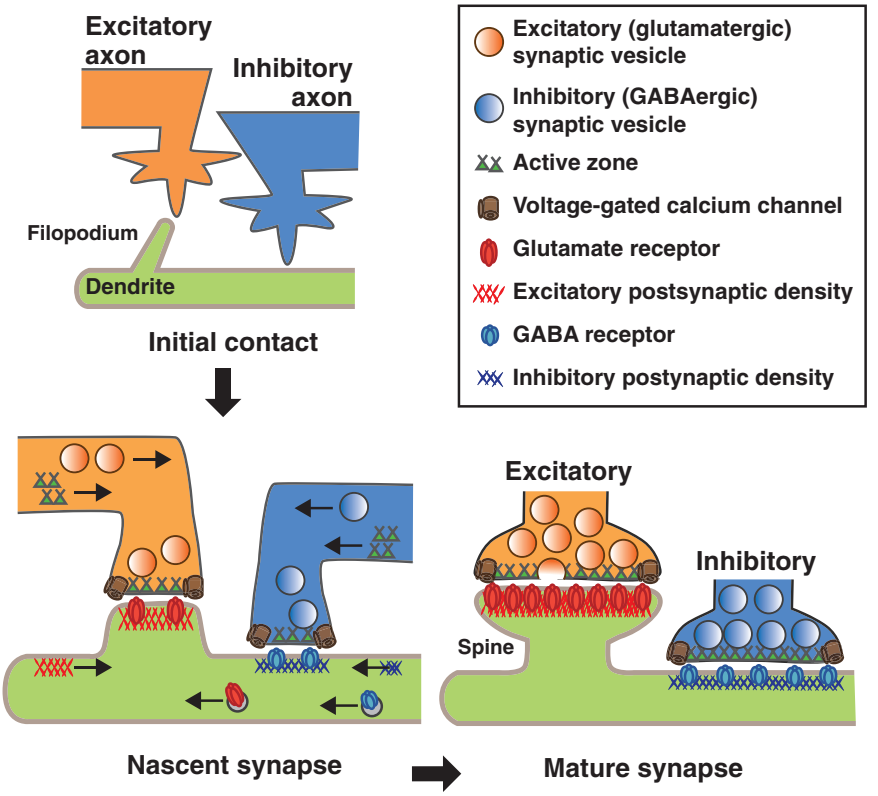

Fig. 1 Synapse formation. Initial contact is established between axons and dendrites. Dendritic filopodia precede the formation of dendritic spines at excitatory synapses. Once initial contact has been established, a nascent synapse may form, at which synaptic components assemble. Presynaptically, active zones and VGCCs assemble in both excitatory and inhibitory cells, and synaptic vesicles containing excitatory (glutamate) or inhibitory (GABA) neurotransmitter accumulate. Postsynaptic excitatory or inhibitory densities containing glutamate or GABA receptors, respectively, form opposite the presynaptic specialization.

domain leading to intracellular catalytic activity (*11*). The overall structure of RPTKs and RPTPs consists of a highly variable ligand-binding extracellular domain and intracellular catalytic domain(s) (Fig. 2A). Upon ligand binding, RPTKs dimerize and the intracellular kinase domains cross-autophosphorylate, increasing their catalytic activity and providing docking sites for associated proteins. This activates downstream signalling cascades. RPTPs appear to act in an opposite manner. Inactive RPTPs are dimerized and reciprocally occlude their phosphatase domains; however ligand binding monomerizes RPTPs, uncovering and thereby activating the catalytic domain (*12*).

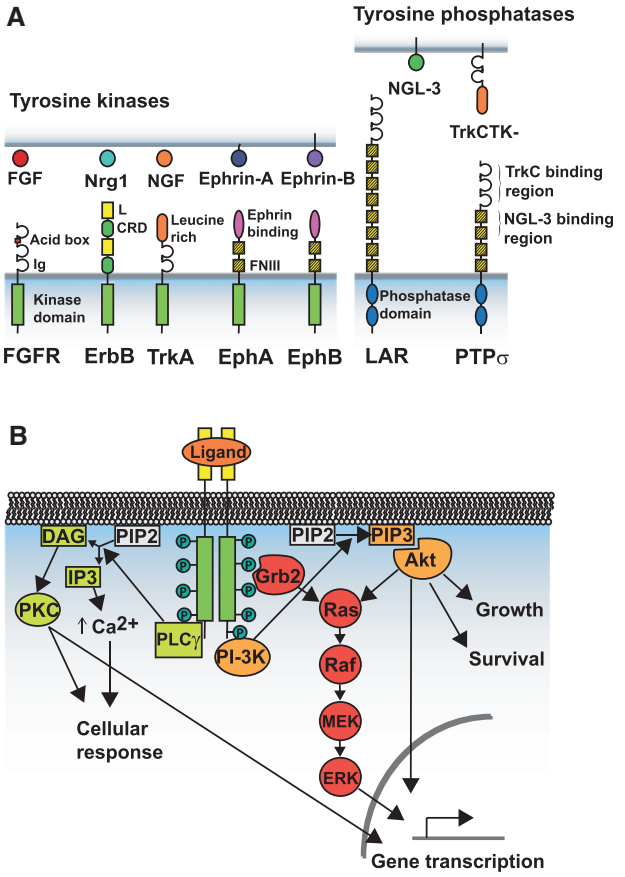

Fig. 2 RPTKs and RPTPs involved in synaptogenesis. (A) Structure of RPTKs and RPTPs described in this review. (B) A simplified sketch of RPTK signalling, demonstrating the 'classic' pathways. Ligand binding causes RPTK dimerization and cross-autophosphorylation, which increases the catalytic activity of the kinase and resultant phosphotyrosine residues serve as docking sites for adaptor proteins. Grb2 is recruited to the RPTK and phosphorylated, causing downstream signalling leading to ERK activation, which phosphorylates numerous transcription factors and induces gene transcription. Phosphatidylinostiol-3 kinase (PI-3K) binds directly to the RPTK and phosphorylates phosphatidylinostol-4,5-bisphosphate (PIP2) to phosphatidylinostol-3,4,5-triphosphate (PIP3), which activates Akt signalling. Phospholipase-C gamma (PLCγ) also binds directly to RPTKs, and cleaves PIP2 to generate diacylglycerol (DAG) and inositol triphosphate (IP3), which separately signal to cause an increase in intracellular Ca^{2+} and activate protein kinase C (PKC). All three pathways interact with each other on various levels. Ig: immunoglobulin domain, L: L domain, CRD: cysteine-rich domain, FNIII: fibronectin type III domain.

Once an RPTK is activated, a number of stereotypic signalling events occur. Phosphorylation of multiple tyrosine residues on the intracellular portion, including the kinase domain of the RPTK, further increases the catalytic activity of the RPTK and makes docking sites for adapter proteins to set off intracellular signalling cascades. Classically, the main signalling readouts were thought of as parallel, linear pathways—the extracellular signal-regulated kinase [ERK, also known as mitogen-activated protein kinase (MAPK)] pathway, the phosphoinositide-3 kinase (PI3K)/Akt pathway and the phospholipase-C gamma (PLCγ)/Ca^{2+} pathway (Fig. 2B). The ERK pathway involves sequential activation of Ras–Raf–MAPK/ERK kinase (MEK)–ERK, and ERK phosphorylates transcription and translation factors, leading to expression of proteins important for cell proliferation and differentiation. PI3K is activated by the RPTK and activates Akt; it also activates Raf, feeding into the ERK pathway. Akt activates dozens of transcriptional, translational and cell metabolism factors, leading to cell survival and differentiation. PLCγ is activated at the RPTK and hydrolyzes phosphatidylinositol to produce inositol triphosphate (IP3) and diacylglycerol (DAG), which increase intracellular Ca^{2+} concentration and gene transcription. One of the pathways may predominate over the others to give a different functional outcome, but it is now being appreciated that the spatiotemporal kinetics of phosphorylation, including numerous positive and negative feedback loops, may play a large part in differentiating outcomes of RPTK activation. An additional level of control is achieved through endocytosis of activated RPTKs (*11*, *13*). For example, endocytosed RPTKs may be mono-ubiquitinated and targeted to the lysosome for degradation, terminating the signal. Endocytosed RPTKs can also be recycled to the membrane, either from early or late endosomes. Furthermore, they can maintain their activity, creating 'signalling endosomes', which can be trafficked to the cell body (*14*).

RPTPs may act to control duration of RPTK signalling (*15*). However, much of the described downstream signalling involves direct recruitment of proteins to the activated RPTP. The majority of RPTPs have two intracellular catalytic domains: the membrane proximal one is catalytically active and the membrane distal phosphatase domain is typically not catalytically active but serves as a binding site to recruit protein partners and control trafficking of the RPTP.

The functions of RPTKs and RPTPs have been described in neuronal survival and nervous system development (*16*, *17*). Now, the roles of RPTKs and RPTPs in synaptogenesis are emerging.

Presynaptic development

The presynaptic specialization forms in the axon terminal at the site where it makes contact with the postsynaptic dendrite. It functions to release neurotransmitter into the synaptic cleft in response to neural activity (*18*). In the presynaptic terminal, a congregation of synapse-specific proteins forms active zones, where neurotransmitter-laden synaptic vesicles

Table I. Pre-synaptic receptors.

Receptor	Ligands involved in synapse differentiation	Region and/or cell type	Developmental stage that the effects were examined	Observed synaptic effects	Demonstrated signalling involved
FGFR2b	FGF7, FGF22	Synapses onto CA3 hippocampal neurons	P8-Adult *in vivo* & 8DIV-21DIV	Decreased glutamatergic and GABAergic SV accumulation in FGF22KO and FGF7KO, respectively. More pronounced decrease in GABAergic than glutamatergic SV clustering in FGFR2KO.	Kinase activity
ErbB4	Nrg1	Cortical PV+ interneurons	P30 *in vivo*	*In utero* expression of Nrg1 in pyramidal cells (at E16.5) leads to increased GAD+ inhibitory boutons on transfected cells and increased mIPSCs.	–
TrkA	NGF	Autonomic sympathetic post-ganglionic neurons	16-18DIV	NGF stimulation of axons 16-18DIV leads to increased PSDs in a retrograde manner within 12h post-stimulation.	PI3K and ERK at axon terminal; retrograde transport of signalling vesicle to dendrite, with ERK signalling
LAR	NGL-3	Hippocampal cells	14-16DIV for overexpression & 10-16DIV for knock-down	Overexpression of NGL-3 (12-15DIV) leads to more presynaptic glutamatergic SV accumulation on transfected dendrites. Knock-down of NGL-3 (10-14DIV) leads to decreased glutamatergic SV clustering and decreased electrophysiologically functional synapses.	–
PTPσ	TrkC	Hippocampal cells	14-15DIV	Overexpression of TrkC (9-10DIV) leads to increased glutamatergic SV clustering in contacting neurons. Soluble TrkC increases glutamatergic SV accumulation.	–

are docked by Ca^{2+}-sensitive SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) proteins. When electrical activity depolarizes the neural membrane, voltage-gated Ca^{2+} channel (VGCC) opening allows Ca^{2+} influx into the nerve terminal, causing the SNARE proteins to fuse synaptic vesicles with the membrane and release their contents into the cleft. Thus, presynaptic assembly requires formation of the active zone and accumulation of synaptic vesicles. In developing cultured neurons, separate pre-formed clusters of synaptic vesicle proteins and active zone components are trafficked along the axon, suggesting that accumulation of active zone components and clustering of synaptic vesicles may require distinct signalling pathways (*19*). Furthermore, diverse synapses have idiosyncratic properties such as different bouton size, release probability and morphology suggesting that distinct signalling mechanisms are required for each synapse. RPTKs and RPTPs appear to contribute to determining such specificity (Table I).

RPTKs in presynaptic differentiation

Several RPTKs control accumulation of synaptic vesicles in developing neurons in cell/synapse specific manners (Fig. 3). FGFR1/2 stimulated by FGF22 leads to glutamatergic synaptic vesicle accumulation in nerve terminals contacting CA3 pyramidal neurons, FGFR2 stimulated by FGF7 leads to GABAergic vesicle accumulation in interneurons contacting CA3 pyramidal cells, and ErbB4 stimulates GABAergic synaptic vesicle accumulation in parvalbumin-positive (PV+) interneurons in the cortex. Presynaptic TrkA has a unique role: TrkA complexes are trafficked within the cell and contribute to postsynaptic development of the same neuron in the autonomic peripheral nervous system.

FGFRs coordinate presynaptic differentiation of excitatory and inhibitory synapses onto CA3 hippocampal neurons. FGFs have an early inductive role in excitatory (glutamatergic) and inhibitory (GABAergic) presynaptic development (*20*). Specifically, FGF7 promotes accumulation of inhibitory synaptic vesicles and FGF22 of excitatory synaptic vesicles, at axon terminals contacting hippocampal CA3 neurons (Fig. 3A and B). When FGFs are transfected into hippocampal cultures, neurotransmitter-specific synaptic vesicle accumulation can be observed by 7DIV. Sections from brains of *Fgf22* and *Fgf7* knock-out (KO) mice display decreases in glutamatergic and GABAergic synaptic vesicle clustering respectively by P8, early stages of

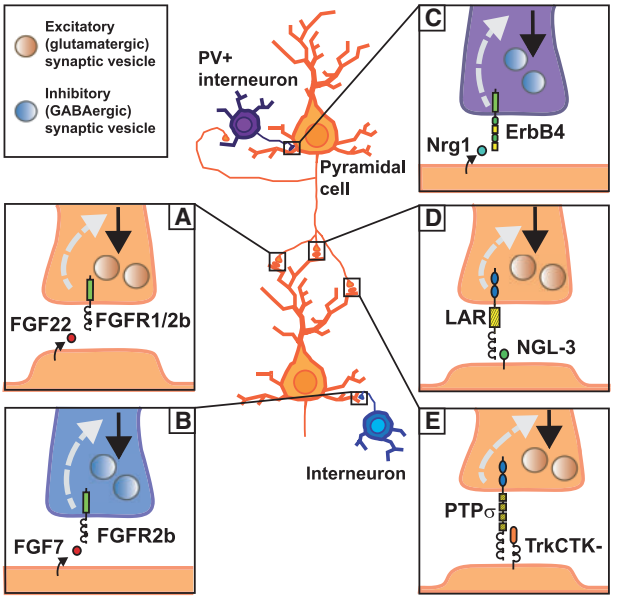

Fig. 3 RPTKs and RPTPs involved in presynaptic differentiation. Orange neurons represent excitatory pyramidal cells, blue neuron represents inhibitory interneuron and purple neuron represents PV+ interneuron. (A, B) FGF22 (A) and FGF7 (B) are secreted from CA3 pyramidal neurons in the hippocampus to induce accumulation of glutamatergic and GABAergic synaptic vesicles, respectively, in contacting axons through FGFR1/2b or FGFR2b. (C) ErbB4 is expressed in PV+ interneurons and responds to Nrg1 secreted from pyramidal neurons, leading to increased inhibitory synapse formation. (D) Stimulation with NGL-3 increases glutamatergic vesicle accumulation through activation of the RPTP LAR. (E) The non-catalytic isoform of TrkC (TrkCTK-) activates PTPσ leading to accumulation of glutamatergic vesicles.

synaptogenesis. This timing suggests that FGF signalling is important for the initial differentiation of synapses formed onto CA3 neurons. Intriguingly, FGFs promote accumulation of synaptic vesicles, but do not increase the number of active zones, suggesting that synaptic vesicle and active zone accumulation are dissociable, at least in the first stages of development.

The differential nature of FGF7 and FGF22 on synapse development begs the question of whether the receptors and downstream signalling are also divergent between excitatory and inhibitory synapses. In mitogenic assays, both FGF7 and FGF22 bind with highest affinity to the b splice form of FGFR2 (FGFR2b). However, FGF22 also binds to FGFR1b with high affinity (*21*), so the differential synaptic effects could be mediated by separate receptors, with FGFR1b relating FGF22 signals (excitatory) and FGFR2b relating FGF7 and FGF22 signals (inhibitory and excitatory). Indeed, the clustering of both glutamatergic and GABAergic vesicles is decreased in FGFR2KO mice, but the decrease is more pronounced at GABAergic than at glutamatergic synapses (*20*), suggesting that the differential effects of FGF22 and FGF7 are at least in part achieved by their use of different sets of receptors (Fig. 3A and B). Analysis of FGFR1KO mice is underway.

Finally, studies in epithelial cells demonstrate that activation of FGFR2b by FGF7 induces clathrin-dependent endocytosis and targets the receptor to degradation through the ubiquitin pathway, whereas activation by FGF10 induces internalization but the receptor is then recycled back to the cell surface (*22*). FGF7, FGF10, and FGF22 are structurally related, but the fact that different ligands (FGF7 and FGF10) can cause different fates of the same receptor suggests that analogously, FGFR2b could be differentially trafficked in response to FGF7 and FGF22.

As for synaptogenic downstream signalling of the FGFRs, there are clues that FGFR2b requires kinase signalling to induce synaptic vesicle accumulation, but not ERK signalling. In an assay to observe synaptic vesicle accumulation in cultured motor neurons, treatment with a tyrosine kinase inhibitor decreases the ability of FGF22 to accumulate synaptic vesicles down to baseline levels, but inhibition of ERK has little effect (*23*). Akt and PLCγ pose as obvious candidate pathway molecules. Alternatively, these 'classic' pathways may be activated and necessary for long-term changes in the cell, such as transcription of genes of differentiation, but different, localized cues cause synaptic vesicle accumulation, such as phosphorylation of membrane-associated proteins to tag synaptogenic sites.

ErbB4 promotes presynaptic bouton formation on PV+ interneurons. ErbB4 is a member of the epidermal growth factor receptor (EGFR) family, and its ligand is neuregulin 1 (Nrg1). ErbB4 is expressed both postsynaptically and presynaptically within one cell type in the cortex—GABAergic, PV+ interneurons (Fig. 3C) (*24–26*). Analysis of conditional KO mice in which the *erbB4* gene is specifically deleted either from interneurons or from pyramidal cells show that ErbB4 protein levels in brain lysate decreased only in the interneuron specific ErbB4 KO. PV+ interneurons, which include basket cells and chandelier cells, form inhibitory synapses onto the soma and axons of pyramidal cells in the prefrontal cortex and hippocampus and have an important role in generating gamma oscillations in the hippocampus and preventing network overexcitability. The evidence for ErbB4 signalling in presynaptic differentiation relies on *in utero* knock down at E16.5 of ErbB4 by shRNA, which results in a decrease in the density of inhibitory boutons at P30 (*25*). The decrease in inhibitory synapses formed by PV+ interneurons was verified with electrophysiological recordings. Furthermore, pyramidal cells that overexpress Nrg1 by *in utero* electroporation display an increase in the number of inhibitory synapses on the cell body and axon by P30, suggesting that Nrg1 from the postsynaptic pyramidal cell signals through ErbB4 at nascent synapses to increase the number of inhibitory synapses onto the pyramidal cell (Fig. 3C). What exactly happens in this broad time window between the electroporation into the embryos and assessment in the mature network at P30, and what signalling pathways are involved, remains to be determined. Nevertheless, the finding that ErbB4 has a specific role in PV+ interneurons in the cortex lends precedence to the idea that RPTKs have similar signalling in organizing the

presynaptic machinery in the context of cell-specific expression.

TrkA controls synaptic differentiation in peripheral autonomic neurons and displays novel mechanisms coordinating intraneuronal pre- and postsynaptic differentiation. An interesting finding concerning TrkA signalling in the autonomic peripheral nervous system expands the potential range of RPTK signalling. Signalling endosomes containing activated TrkA receptor are transported retrogradely from axon terminals to dendrites of the same neuron, suggesting that RPTK signalling can have far-reaching effects (*27*). Post-ganglionic, noradrenergic neurons receive inputs from preganglionic, cholinergic neurons and make contacts with target organs in the periphery. Nerve growth factor (NGF) derived from peripheral organs supports survival of noradrenergic neurons and increases their presynaptic elaborations at the axon terminals contacting the peripheral organs in a TrkA-dependent manner (*28*). However, the periphery-derived NGF also increases the number of cholinergic synapses on the dendrites of noradrenergic neurons (*27*). This retrograde effect of NGF was thoroughly demonstrated by separating the axon terminals from the dendrites and cell bodies by using a microfluidic compartment system. Application of an NGF blocking antibody in the distal axons leads to a decrease in postsynaptic specializations in the dendrites. The most stunning finding is that the TrkA receptor signalling endosomes are back-trafficked from the axons to the dendrites. NGF-mediated induction of postsynaptic specializations in the dendrites requires separate signalling in axons and dendrites—PI3K signalling in the axons but not the dendrites, and ERK signalling in both the dendrites and axons. TrkA signalling endosomes might be important for cholinergic synapses in the CNS as well, since application of NGF blocking antibodies decreases cholinergic boutons in the cortex (*29*). Whether a similar mechanism of retrograde signalling from the presynaptic axon terminal to postsynaptic specialization in the dendrites applies to other RPTKs in other neural cells remains to be determined.

Receptor protein tyrosine phosphatases in presynaptic development

There are eight families of RPTPs (*12*) but only one family, type IIa RPTPs, has been shown to play a role in inducing synapse formation. The three members of this family are PTPδ, PTPσ and LAR. The three RPTPs are expressed in distinct but non-overlapping areas of the brain, with higher expression levels at P7 to P14, a time of heightened synapse development (*30*). All three have been found to have a presynaptic role in synaptic vesicle accumulation *in vitro* in response to postsynaptic membrane-bound protein netrin-G ligand 3 (NGL-3) (Fig. 3D). Furthermore, PTPσ and LAR can have a bidirectional effect, signalling back through NGL-3 to cause PSD formation. The receptor PTPσ also bidirectionally interacts with TrkC (Fig. 3E) (*31*).

LAR controls presynaptic differentiation at excitatory synapses in hippocampal neurons. When the presynaptic role of LAR was first discovered in *Drosophila* and *Caenorhabditis elegans*, it was found to organize active zones, based on the observation that active zones are expanded and sparser in *Lar−/−* mutants, with a normal number of synaptic vesicles per synapse (*32*, *33*). However, as of yet, the role of LAR in active zone formation has not been confirmed in vertebrates. Instead, LAR has been shown to have a role in presynaptic vesicle accumulation. Overexpression of NGL-3 in hippocampal cells transsynaptically binds to presynaptic LAR (as well as PTPδ and PTPσ) in hippocampal cultures, stimulating the accumulation of glutamatergic synaptic vesicles, but not that of GABAergic synaptic vesicles on the NGL-3-expressing dendrite (Fig. 3D) (*30*, *34*). Increased synaptic vesicle accumulation in axons contacting NGL-3-expressing HEK cells can be partially blocked by addition of a soluble LAR ectodomain, suggesting that NGL-3 acts in part through LAR to transsynaptically stimulate synapse differentiation. It is not clear if the presynaptic role of LAR requires phosphatase activity to accumulate synaptic vesicles in the axon terminal.

Presynaptic PTPσ promotes excitatory synapse formation through interactions with TrkC. A novel synaptogenic interaction has recently been identified from an unbiased expression screen in cultured neurons between the phosphatase receptor PTPσ and TrkC, a well-described receptor tyrosine kinase for neurotrophins (*31*). The two signal in a bidirectional manner, with PTPσ expressed presynaptically and TrkC postsynaptically (Fig. 3E). TrkC interacts with neurotrophin-3 (NT-3), which activates its intracellular kinase; however, PTPσ binds at a different site of TrkC, which induces postsynaptic differentiation in a kinase-independent manner, serving more as a cell adhesion molecule. Likewise, distinct sites on PTPσ are required for TrkC and NGL-3 binding (Fig. 2A). The intracellular domain of PTPσ is necessary for presynaptic vesicle clustering; however, it is not shown whether this requires the phosphatase catalytic activity. Since LAR and PTPσ are related and both can promote vesicle accumulation at excitatory synapses, it is possible that they act through similar mechanisms. The later timing of both LAR and PTPσ effects (transfections at 12DIV for LAR, 9-10DIV for PTPσ studies, both assessed at 15DIV) suggests that these RPTPs may act to coordinate later maturation steps through assembly of presynaptic proteins in nascent synapses.

Postsynaptic development

At the synaptic site where an axon terminal contacts a dendrite (in some cases the cell body or axon), a postsynaptic specialization forms. The most prominent feature is the postsynaptic density (PSD), a protein mesh that clusters the postsynaptic proteins at the synapse. Additionally, many excitatory, but not inhibitory synapses are formed onto dendritic spines, which are

Table II. Post-synaptic receptors.

Receptor	Ligands involved in synapse differentiation	Region and/or cell type	Developmental stage that the effects were examined (treated → observed)	Observed synaptic effects	Demonstrated signalling involved
ErbB4	Nrg1	Cortical PV+ interneurons	5→7DIV, 12→14DIV: size and number 19→21DIV: size	Nrg1 increases number and size of excitatory neurons formed on PV+ interneurons.	Tyrosine kinase, proteasome
EphB2	ephrin-B2	Cortical cells	NMDAR clustering: 1DIV AMPAR clustering: 4→7DIV Ephexin5: 10→14DIV Cofilin activity: 12→14DIV	Ephrin-B2 treatment of cultured cells increases number of excitatory synapses.	Ephexin5, RhoA, Fyn, FAK, PAK, CREB
EphA4	ephrin-A1	Organotypic hippocampal slices	18→20DIV	Ephrin-A1 treatment decreases number of dendritic spines.	Cdk5, Ephexin1, RhoA
EphA5	ephrin-A5	Hippocampal cells	1→6DIV 1→18DIV	Ephrin-A5 treatment of cultured cells increases excitatory synapses.	VGCCs, Ca^{2+}, PI3K, cdc42, CREB
LAR	–	Hippocampal cells	13→18DIV	Knock-down of LAR decreases number of dendritic spines.	Tyrosine phosphatase activity

thought to create a unique signalling microenvironment for more efficient postsynaptic effects (*35*). Synapse formation entails clustering of PSD proteins, such as PSD-95 and gephyrin at excitatory or inhibitory synapses, respectively, formation of dendritic spines from dendritic filopodia, and clustering of appropriate neurotransmitter receptors and ion channels. A long and thin spine is more filopodia-like and immature, while mushroom-shaped spines are more stable and mature. The repertoire of receptors changes over development: e.g. NMDA-sensitive glutamate receptors (NMDARs) are present from the early stages of excitatory synapse formation, and the insertion of AMPA-sensitive glutamate receptors (AMPARs) signifies excitatory synapse maturation (*36*).

RPTKs that are shown to be involved in excitatory postsynaptic differentiation include ErbB4, EphB2, EphA5 and EphA4 (Table II). Significantly, these RPTKs have distinct roles, acting at different synapses or mediating differential effects with the use of distinct signalling pathways (Fig. 4A–D). Meanwhile, type IIa RPTPs (LAR, PTPσ and PTPδ) are involved in activity-dependent postsynaptic differentiation (Fig. 4E).

Receptor protein tyrosine kinases in postsynaptic development

Four RPTKs have been shown to control an array of divergent processes at excitatory synapses, including PSD protein assembly, dendritic filopodial motility, synapse maturation or elimination. Importantly, each molecule appears to have unique roles: ErbB4 plays a role at cortical excitatory synapses onto PV+ interneurons, EphB2 increases filopodial motility and synapse number at hippocampal excitatory synapses, EphA5 increases hippocampal dendritic spines and EphA4 inhibits dendritic spine stabilization and decreases synapse number at hippocampal excitatory synapses (Fig. 4A–D).

ErbB4 controls PSD stability in PV+ interneurons. In addition to its role in presynaptic differentiation at axon terminals of PV+ interneurons described above, ErbB4 also has a role in postsynaptic differentiation in the dendrites of PV+ interneurons (*26*). ErbB4 controls two aspects of postsynaptic development—quantity and maturity of excitatory PSDs in the inhibitory neurons. Application of the ErbB4 ligand Nrg1 to cultured cortical neurons at earlier stages of maturity, 5DIV and 12DIV, results in an increase in number and size of PSDs within 2 days. However, application of Nrg1 at 19DIV only increases PSD size, not number, suggesting its contributions to maturation but not induction of synapses at later stages. Furthermore, *in utero* knock-down of ErbB4 in interneurons leads to a decreased number of excitatory inputs to these neurons at P30 in the mature network, attesting to ErbB4's role in postsynaptic cells *in vivo* (*27*). Activated ErbB4 stabilizes postsynaptic proteins and halts their constitutive turnover. Treatment of cortical cells with a protein synthesis blocker decreases the number and size of PSDs over time. However, addition of Nrg1 to these cultures stabilizes the PSDs and prevents their turnover. Furthermore, this requires kinase-active ErbB4, as pharmacological treatment with a kinase inhibitor interferes with ErbB4-mediated stabilization of PSDs, and co-transfection PSD-95 and a kinase-dead ErbB4 mutant into HEK cells also fails to prevent PSD-95 turnover as compared to a kinase-active ErbB4. These experiments suggest that ErbB4 acts throughout synapse development to increase stability of postsynaptic proteins at emerging or preformed synapses (Fig. 4A). Stabilization of proteins in the PSD increases both the strength and number of synapses

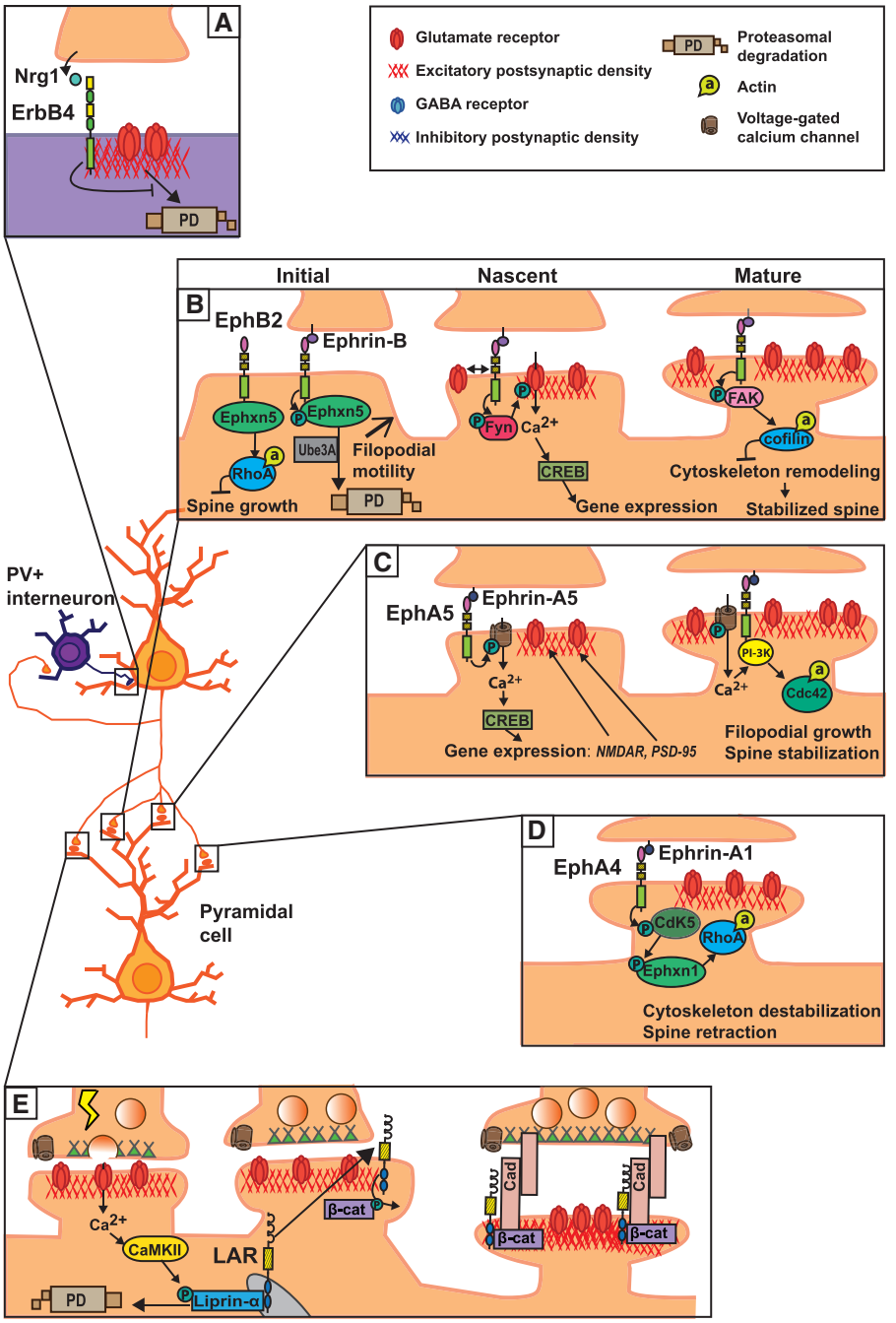

Fig. 4 RPTKs and RPTPs involved in postsynaptic differentiation. Orange neurons represent excitatory pyramidal cells and purple neuron represents PV+ interneuron. (A) Nrg1 secreted from pyramidal neuron activates ErbB4 expressed in PV+ interneuron. ErbB4 activity prevents degradation of PSD proteins, leading to stabilization of synapses. (B) Ephrin-B-stimulated EphB2 increases dendritic filopodial motility (left). Without ephrin-B binding, EphB2 binds to ephexin5 (Ephxn5), which activates RhoA to stabilize the actin cytoskeleton and inhibit spine growth. Ephrin-B-stimulated EphB2 phosphorylates ephexin5, which targets ephexin5 for degradation through ubiquitin protein ligase Ube3A; lack of RhoA activation destabilizes actin cytoskeleton and leads to filopodial motility. EphB2 also induces NMDAR clustering in the PSD through direct interactions (middle). Phosphorylation of the NMDAR through Fyn kinase increases permeability to Ca^{2+} and leads to increased CREB activation and gene transcription. In a mature spine (right), EphB2 activation activates focal adhesion kinases (FAKs) and stabilizes cytoskeleton through RhoA/cofilin suppression of actin cytoskeleton remodeling. (C) Ephrin-A5-activated EphA5 leads to phosphorylation of VGCCs and increased Ca^{2+} permeability, leading to increased expression of NMDARs and PSD-95 through CREB transcription factor (left). Intracellular Ca^{2+} activates PI-3K, which leads to filopodial growth and spine stabilization through cdc42 (right). (D) Ephrin-A1-stimulation decreases excitatory synapse number through EphA4 activation. EphA4 phosphorylates Cdk5, which then phosphorylates ephexin1 (Ephxn1) leading to destabilization of cytoskeleton through RhoA. (E) Synaptic activity activates CaMKII and leads to phosphorylation of liprin-α, which targets liprin-α for degradation (left). Degradation of liprin-α releases LAR from Golgi membrane and allows its translocation to synapse (middle). LAR dephosphorylates β-catenin (β-cat), which binds to distal phosphatase domain of LAR. β-catenin and cadherin (Cad) interactions increase PSD clustering (right).

onto PV+ interneurons, thus increasing the amount of excitatory input received by these cells.

EphB2 coordinates multifarious aspects of excitatory synapse formation. EphB2's polyphonic contributions to postsynaptic maturation encompass both neurotransmitter receptor clustering in the PSD and control of cytoskeletal dynamics in the dendritic filopodia and spines (Fig. 4B). Postsynaptic EphB2 is activated by presynaptic ephrin-B. Ephrin-B is a transmembrane protein, and the interaction between EphB and ephrin-B promotes synapse formation in a bidirectional manner.

In the nascent synapse, EphB2 helps cluster NMDARs directly and AMPARs indirectly. EphB2 directly interacts with NMDARs through their extracellular domains in a cis manner in response to ephrin-B-binding to EphB2 (Fig. 4B, centre) (*37*). Intracellularly, EphB2 phosphorylates the NR2B (also known as GluN2B) subunit of NMDARs, possibly through the non-receptor tyrosine kinase Fyn (*38*). The phosphorylated NMDAR has enhanced Ca^{2+} influx in response to glutamate. This Ca^{2+} influx leads to Ca^{2+}-dependent activation of CREB (cAMP responsive element binding protein) transcription factor and transcription of immediate early genes, including *c-fos*, *BDNF* and *cpg15*, which contributes to maturation of the neuron (Fig. 4B, centre). At the same time, ephrin-B-activated EphB2 recruits AMPARs to the nascent synapse through clustering of PSD proteins (*39*). Kinase activity is required for AMPAR containing PSDs to be formed at the tips of the dendritic spines; without EphB2 kinase activity, AMPARs and PSD proteins fail to localize to the tip of the dendritic spine and instead remain on the dendritic shaft.

The second important aspect of postsynaptic EphB2 is its control of filopodial motility. An increase in filopodial motility may lead to an increase in the number of synapses formed through an increased probability of contact between the axon and dendrite. Knock out of EphB2 in neurons leads to a decrease in both filopodial motility and number of synapses formed starting at 10DIV, during the peak of synaptogenesis (*40*). Transfection experiments with a kinase-dead EphB2 and a constitutively active form of PAK (p21 activated kinase), which is a downstream target of EphB2, demonstrate that one or the other is not sufficient to rescue synapse formation in EphB2KO neurons, but both together can increase synapse number. The constitutively active PAK alone can increase filopodial motility without increasing synapse formation, suggesting that filopodial motility and synapse number are linked but dissociable processes.

Finally, EphB2 also controls actin stability through the RhoA guanine-exchange factor (GEF) ephexin5 (Fig. 4B, left) (*41*). The small GTPase RhoA stabilizes actin. Ephexin5 facilitates RhoA action by increasing RhoA turnover to its active (GTP-bound) form. Beginning at 2DIV, ephexin5 is found bound to EphB2 in dendrites. Ephrin-B binding activates EphB2, and activated EphB2 phosphorylates ephexin5. This marks ephexin5 to be tagged for degradation by a ubiquitin protein ligase Ube3A. Degradation of ephexin5 slows turnover of RhoA to its active form and has the effect of destabilizing actin filaments and increasing filopodial motility, resulting in an increase in synapse density (Fig. 4B, left). Later in synaptogenesis, at 14DIV, the manner in which EphB2 controls actin dynamics changes. Activated EphB2 phosphorylates focal adhesion kinase (FAK), which activates RhoA and cofilin to control actin cytoskeleton dynamics and promote the stabilization of a mature mushroom-shaped spine (Fig. 4B, right) (*42*). Thus, the role of EphB2 changes with the context of the maturity of the synapse, from increasing filopodial motility in early synaptogenesis to stabilizing the mature spine.

EphA5 promotes excitatory synapse formation through different pathways from EphB2. Treatment of hippocampal neurons with ephrin-A5 activates EphA5 receptors and leads to an increased number of excitatory synapses (*43*). The general aspects of synaptogenic signalling downstream of EphA5 are similar to those of EphB2: in both, increases in Ca^{2+} influx lead to activation of CREB transcription-factor and gene expression, and in both the RPTK signalling leads to remodeling of the actin cytoskeleton and filopodial motility/spine maturation. However, the EphA5 and EphB2 pathways utilize different downstream molecules—(i) EphB2 phosphorylates NMDA receptors to increase Ca^{2+} influx (Fig. 4B, middle), while EphA5 phosphorylates VGCCs (Fig. 4C, left) and (ii) EphB2 remodels the actin cytoskeleton through control of RhoA (Fig. 4B), whereas EphA5 controls cdc42 (Fig. 4C, right). It will be interesting to examine whether the two Eph receptor pathways act in different synapses and/or they control temporally different aspects of differentiation at the same synapse.

Another important aspect that emerges from this study (*43*) is that EphA5 increases the expression of NMDA receptors and PSD-95 (Fig. 4C, left), strengthening the PSD through long-range effects.

EphA4 eliminates excitatory synapses through control of actin cytoskeleton dynamics at later stages of synaptogenesis. In contrast to EphB2 and EphA5, the receptor EphA4 is a negative regulator of excitatory synapse development in response to ephrin-A1 stimulation. In more mature cultured hippocampal cells, addition of eprhin-A1 at 18-20DIV decreases the number of dendritic spines (*44*). EphA4 phosphorylates cyclin-dependent kinase 5 (Cdk5), and Cdk5 phosphorylates the ephexin1 (another RhoA GEF) and activates it. However, in contrast to ephexin5-RhoA dynamics, activation of ephexin1 and subsequent cycling of RhoA into its active form leads to spine retraction (Fig. 4D). Thus, EphA4 is a late stage negative regulator of synaptic spines, which could function to prevent synapse formation at inapposite sites, to decelerate synapse formation and/or prune back extraneously formed synapses. The opposing effects of EphA4 and EphB2 through the ephexin family proteins may illustrate the temporal control of changing intracellular context in which these molecules function.

Receptor protein tyrosine phosphatases involved in postsynaptic development

Three types of IIa RPTPs (LAR, PTPσ and PTPδ) involved in presynaptic differentiation have been shown to have a postsynaptic role as well.

Activity-dependent postsynaptic LAR localization contributes to late stages of synaptogenesis. The activity-dependent delivery of LAR to synapses supports the role of LAR in later stages of synaptic development. LAR-interacting protein-related protein-α (liprin-α) is involved in localizing LAR to dendritic spines in an activity-dependent manner (Fig. 4E) (*45*). Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) is a Ca^{2+}-sensitive serine/threonine kinase prominently expressed in the PSD of excitatory synapses. It is activated by Ca^{2+} influx in response to synaptic activation. Activated CaMKII phosphorylates liprin-α and leads to its degradation (Fig. 4E, left). Liprin-α binds to the distal phosphatase domain of LAR and is involved in trafficking of LAR, but degradation of liprin-α could release LAR into the active synapses. Released LAR may then reach the surface of the membrane and contributes to assembly of the PSD (Fig. 4E, middle).

LAR, and the closely related PTPδ and PTPσ, are enriched in PSD fractions in the brain. RNAi knock downs of LAR, PTPδ or PTPσ demonstrate a decrease in the number of dendritic spines and excitatory synapses. Expressing dominant-negative forms of LAR that are unable to bind to liprin-α, lack phosphatase activity, or are not properly cleaved from the precursor form decreases the number of synapses in hippocampal cultures. Similarly, dendritic spine density decreases with transfection of the liprin-α or LAR construct that is unable to bind to each other. Furthermore, LAR promotes postsynaptic differentiation through recruitment of β-catenin and cadherins (Fig. 4E, right) (*46*, *47*). In neurons overexpressing dominant-negative forms of LAR, the distribution of β-catenin is altered, with less accumulation to spines. β-catenin is a direct substrate for LAR phosphatase and accumulates to spines in its dephosphorylated form. Thus, LAR is proposed to localize cadherins to synapses through β-catenin dephosphorylation (Fig. 4E, middle-right).

Discussion

Receptor tyrosine kinases and phosphatases have diverse actions in synapse development. Once contacts are formed, presynaptic RPTKs and RPTPs are important for synaptic vesicle accumulation and active zone formation. Despite overlapping presynaptic function, different presynaptic receptors appear to have synapse-specific roles (Fig. 3): FGFR1/2b act at excitatory and FGFR2b at inhibitory synapses contacting CA3 pyramidal cells, ErbB4 is only expressed in PV+ interneurons in the cortex, and LAR promotes excitatory synapses on hippocampal cells. The presynaptic cellular signalling cascades that lead to synaptic vesicle accumulation and active zone assembly are important future topics to address. Postsynaptically, it is possible that different receptors contribute to differentiation of specific synapses as well. For example, ErbB4 also has a postsynaptic role specifically in PV+ interneurons. However, whether other RPTKs and RPTPs are uniquely involved in subtypes of excitatory synapses has yet to be demonstrated. Howbeit, recent studies revealed that postsynaptic RPTKs and RPTPs regulate unique aspects of postsynaptic development at different temporal points in synapse development (Fig. 4). An extreme example is the opposite outcome of EphB2 and EphA4 signalling on excitatory synapse number. Furthermore, postsynaptic signalling pathways and their unique downstream molecules are much better characterized than presynaptic signalling pathways, if only to take example from ephexin1 and ephexin5 downstream of EphA4 and EphB2, respectively. Finally, some of the postsynaptic RPTK and RPTP signalling events are activity dependent; for example, LAR expression at the cell surface, EphB2-regulated transcription, or expression of BDNF and its regulation of inhibitory synapse formation (*38*, *45*, *48*).

How are receptors that control tyrosine phosphorylation different from other receptors involved in synapse formation?

A variety of molecules coordinate synapse formation between neurons, but few have shown to have such pronounced effects both locally (through signalling) and globally (through transcriptional control) as RPTKs and RPTPs described here. Some molecules induce synapses through purely localized effects—an extreme example are pentraxins, which are secreted into the extracellular space and directly bind to and cluster AMPA receptors in the synapse (*49*). Many synaptogenic cell adhesion molecules appear to predominantly act locally through protein–protein interaction. Thus, RPTKs represent unique bimodal effectors that can differentiate both local synapses and entire neurons. Further identification of downstream signalling molecules and target genes of synaptogenic RPTKs and RPTPs would clarify the molecular steps involved in their local and global effects.

What is the role of RPTK-mediated transcriptional control in synapse formation?

RPTKs have well-described roles in initiation of transcription through the ERK pathway and PI3K pathway, that are important in cell survival and differentiation (*17*). The role of transcriptional regulation in synapse formation is implied, but few studies have directly addressed this. It will be interesting to see if RPTK-controlled transcription has a different repertoire of targets compared to other developmental processes.

An important transcriptional control by RPTKs in synapse-bearing neurons is the CREB transcription factor. CREB is phosphorylated by ERK and thus serves as a readout of RPTK signalling, but it can also be phosphorylated by CaMKII, which is activated by activity-driven Ca^{2+} influx into neurons, thus making CREB a good readout for the activity state as well. These two pathways—RPTK and

activity-dependent—may converge and mutually amplify each other. Loss of RPTK signalling may decrease neuronal response to activity-dependent stimuli, thus further affecting synapse and neuronal differentiation in an indirect manner. In addition to direct activation of CREB through ERK, EphA5 can phosphorylate Ca^{2+} channels and increase the probability of voltage-sensitive opening (*43*), and EphB2 phosphorylation of the NMDAR increases its Ca^{2+} permeability (*38*), leading to CaMKII-dependent phosphorylation of CREB. CREB activation leads to transcription of BDNF, among other immediate-early genes, and BDNF regulates inhibitory synapse development (*48*). Thus, RPTK-mediated transcription modulates activity-dependent transcriptional changes and may help establish appropriate and balanced synaptic network.

Do RPTKs act as sensors of synapse development in a neural network?

Interneurons in the brain are locally projecting inhibitory neurons that control the balance of neural network. An intriguing finding is that ErbB4 in PV+ interneurons is localized at both the PSDs opposed to incoming excitatory inputs, and at the presynaptic terminal of the inhibitory output. Two interesting possibilities can explain the role of this dual localization of ErbB4 within PV+ interneurons. One is that the role of ErbB4 in presynaptic development could be a consequence of its role in postsynaptic development, a result of increased Nrg1-induced inputs from pyramidal neurons into PV+ interneurons, driving the formation of inhibitory outputs onto pyramidal cells in an Nrg1–ErbB4-dependent manner. Alternatively, ErbB4 could be acting as a sensor of network maturity and adjusting the transcriptional program of interneurons accordingly. In this model, presynaptic and postsynaptic ErbB4 in interneurons could detect the maturation state of contacting neurons based on Nrg1-mediated ErbB4 activation. Thus, ErbB4-mediated signals may induce transcriptional cues that could control the balance between the input and output of the interneurons leading to appropriate neural network development.

Do RPTKs cross talk with each other by forming heterocomplexes?

A putative interaction between FGFRs and the EphA4 receptor has been reported, in which the two receptors cross-phosphorylate and bind to each other, then synergistically act on downstream signalling through the FGFR adapter protein FRS2 and the RhoA GEF downstream of EphA4, ephexin1 (*50*, *51*). As described above, ephexin1 has a postsynaptic role in dendritic spine retraction in response to EphA4 receptor stimulation (*44*). Recently, an unbiased screen in *Drosophila* identified ephexin as part of a presynaptic signalling cascade, which signals through Eph receptors to VGCCs and is important in synaptic homeostasis of synaptic vesicle release (*52*). It remains to be determined whether ephexins have a presynaptic role in vertebrate CNS synapses, but direct phosphorylation of ephexin by FGFR and subsequent modulation of Ca^{2+} channel signalling could represent one possible mechanism for controlling differentiation of presynaptic terminals containing both FGFR and EphA4. Now that a conditional EphA4 KO mouse has been generated (*53*), precise contributions of EphA4 to synaptogenesis and its putative interaction with FGFRs can be studied with greater accuracy. FGFRs also interact with cell adhesion molecules implicated in synapse formation/maintenance, such as NCAM, L1, N-cadherin and neurophilin (*54*). Such interactions may play a role in coordinated regulation of synapses with different sets of synaptogenic molecules.

Implication in neurological disorders

RPTKs are widely distributed and have broad signalling pathways, but they appear to have very specific and important roles in the formation of synapses. Evidence is accumulating that their dysregulation can lead to profound neurological disorders. For example, genetic studies have linked ErbB4/Nrg1 to schizophrenia (*55*). *Fgf7* KO mice are susceptible to seizures, suggesting that understanding downstream FGFR signalling may lead to insights or therapeutics for epilepsy (*20*). Ube3A, the ubiquitin ligase that acts downstream of EphB2 in control of dendritic spines, is linked to Angelman's syndrome, a genetic disorder characterized by developmental delays and seizures (*41*, *56*). Understanding the dynamics of downstream signalling from RPTKs will help us understand how modulations of specific signalling molecules could lead to synaptic pathologies, either in response to the environment or due to polygenic variation. Many important and enticing questions still remain to be answered about the mechanisms by which RPTKs and RPTPs lead to synapse formation.

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Conflict of interest

None declared.

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