

# Central synapse and neuromuscular junction: same players, different roles

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**The central synapse in the brain and the neuromuscular synapse between motor neuron and muscle fiber are structurally and functionally distinct. Recent studies, however, have identified the localization and potential functions of signaling molecules common to both types of synapse. Strikingly, many of these molecules also have important roles in axon guidance and neurite outgrowth. Among these molecules are the cyclin-dependent kinase Cdk5, agrin, the agrin receptor MuSK, ephrin and the ephrin receptor Eph. Here we summarize the recent findings illustrating the diverse functions of these multi-talented molecules.**

The synapse is a specialized structure that is fundamental to the functioning of neurons. It is highly dynamic and undergoes re-organization in response to various extracellular signals, which forms the basis of synaptic plasticity (i.e. modulation of the synaptic transmission efficacy in response to stimulation). One specific type of synapse that is especially well studied is the neuromuscular junction (NMJ), a specialized structure that is composed of the presynaptic motor nerve terminal, the postsynaptic muscle fiber, and the terminal Schwann cell. Another type of synapse is the central synapse, which forms the connections between different neurons in the central nervous system (CNS) and is generally regarded as functionally more complex than the NMJ. Interestingly, despite their apparent differences in structure and function (Fig. 1), there is growing evidence that some molecules involved in regulating their formation and functioning are common to both types of synapse. These include ion channel (NMDA receptors) and the corresponding scaffold protein (PSD-95), enzyme (nitric oxide synthase) and receptor for neurotrophic factor (TrkB) (reviewed in Ref. [1]). Strikingly, some of the common molecules were originally identified as major players in axon guidance and neurite extension during nervous system development. These include cyclin-dependent kinase5 (Cdk5), which belongs to the Cdk family, and ephrins, which activate Eph receptors, the largest family of receptor tyrosine kinases (RTK) (reviewed in Ref. [2,3]). Conversely, molecules first described as having central roles at the NMJ have recently been demonstrated to participate in synaptogenesis and neurite extension in the developing brain. It is best illustrated by the proteoglycan

agrin. Agrin and its putative receptor MuSK have a well-established role in the formation of the NMJ. Recent evidence, however, suggests a novel function of agrin in neuronal synaptogenesis and, perhaps unexpectedly, neurite outgrowth. This article focuses on the synaptic functions of Cdk5 and Eph receptors, as well as the functional roles of agrin outside the NMJ.

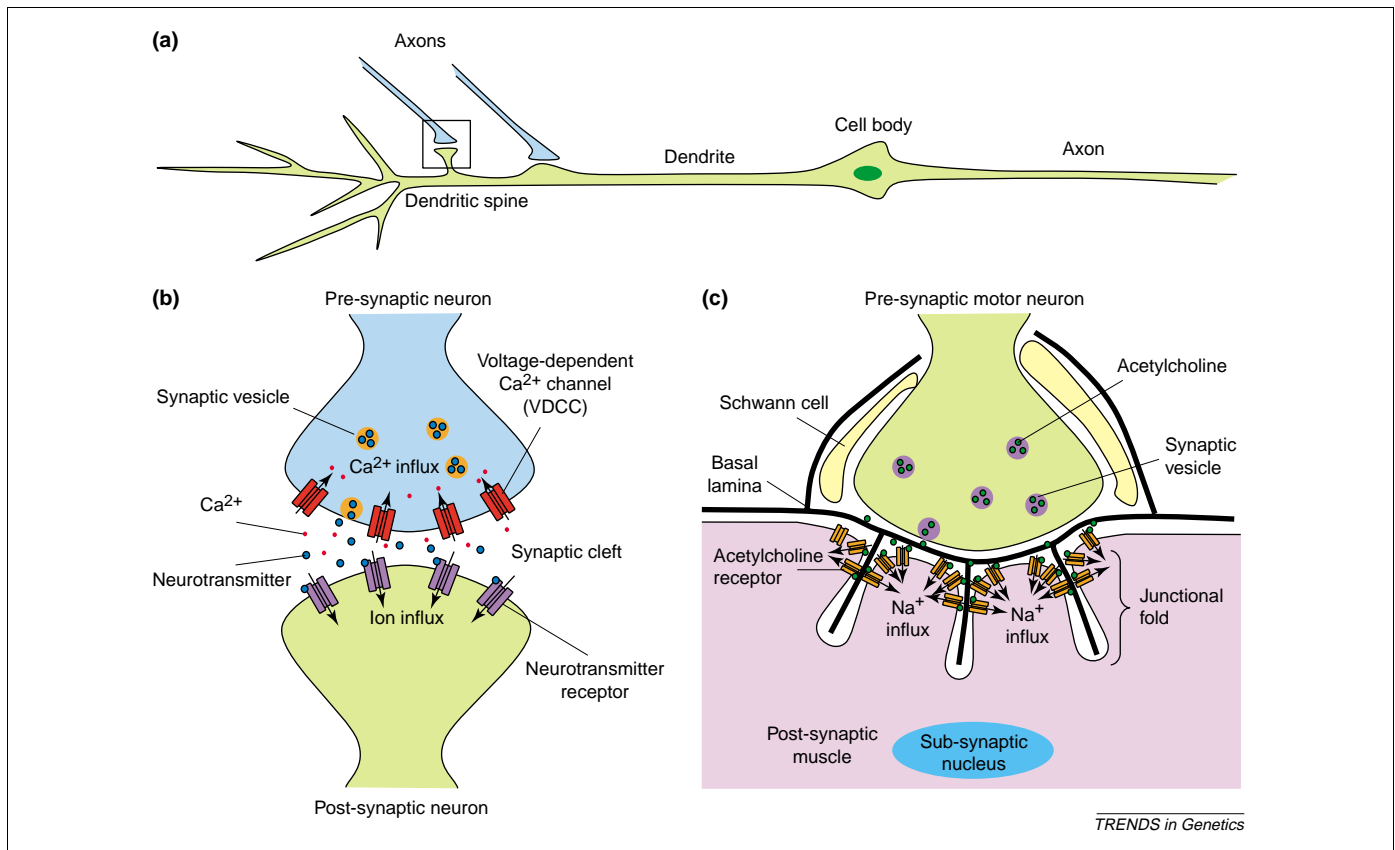
## Involvement of Cdk5 in synaptic functions

Cdk5, a serine/threonine kinase, belongs to the family of cyclin-dependent kinases. Unlike other members of the Cdk family, Cdk5 is not involved in cell-cycle control, and its function is not regulated by cyclins. Rather, the activity of Cdk5 depends on its two activators, p35 and p39. Although the expression of Cdk5 is widespread in different tissues, the kinase activity of Cdk5 is most prominent in the central nervous system (CNS), owing to the restricted expression pattern of its two activators (reviewed in Ref. [2]). Early studies focused on the significance of Cdk5 in neuronal migration and axon guidance, but the role of Cdk5 in modulating synaptic functions in the brain has only recently begun to be unraveled (Fig. 2a).

Cdk5, p35 and p39 are present in the synaptosomes, which form the enriched synaptic membrane fraction of the brain after subcellular fractionation [4]. Immunogold labeling of brain sections reveals the localization of Cdk5 and p39 in both the pre- and postsynaptic regions of the neurons [4,5]. Cdk5 phosphorylates the P/Q-type voltage-dependent calcium channel (VDCC) at the presynaptic terminus, which is responsible for the entry of  $Ca^{2+}$  ions in response to depolarization, and thereby initiates the release of neurotransmitter. The phosphorylation of VDCC in turn inhibits its interaction with the SNARE proteins SNAP-25 and synaptotagmin, which is required for efficient neurotransmitter release [6]. Moreover, roscovitine, an inhibitor of Cdk5, Cdk1 and Cdk2 [7], induces glutamate release from synaptosomes in response to depolarization, further indicating a regulatory role for Cdk5 in the release of neurotransmitter [6].

In postsynaptic neurons, Cdk5 can also modulate neurotransmission. DARPP-32, a phosphoprotein critical in modulating neurotransmission, can act as inhibitor of either protein kinase A (PKA) or protein phosphatase 1 (PP1). Upon phosphorylation by Cdk5 at threonine 75, DARPP-32 inhibits PKA, which in turn attenuates the phosphorylation of PKA substrates, such as the GluR1 subunit of the AMPA receptor [8]. AMPA receptor is one of

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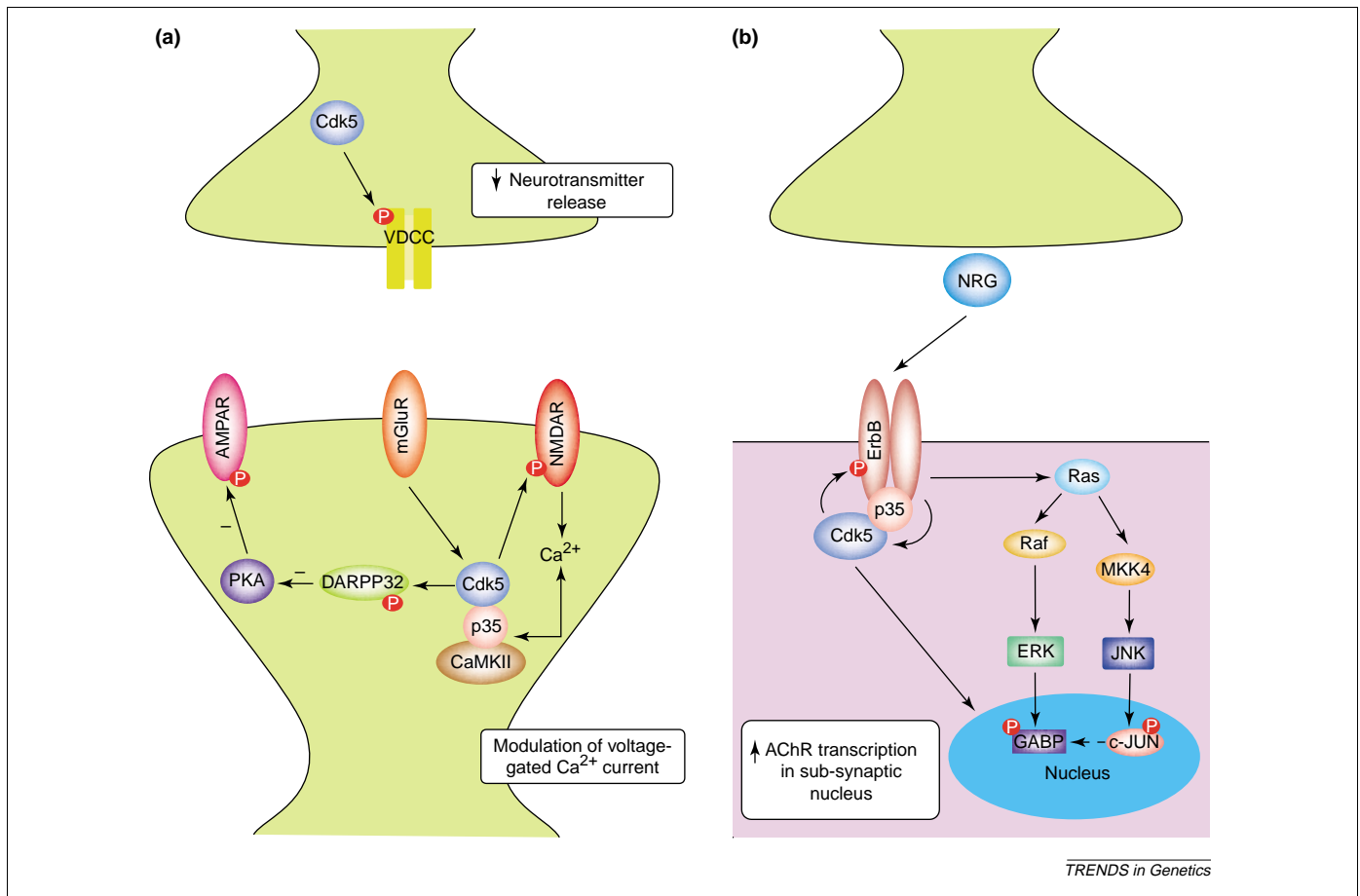
**Fig. 1.** The central synapse in the brain and the neuromuscular junction (NMJ) formed between motor neuron and skeletal muscle. (a) Morphology of a neuron. Two kinds of neuritic processes, axon and dendrite, are extended from the cell body. The axon is responsible for sending out signals to other neurons, whereas the dendrite is involved in receiving signals from other neurons. During nervous system development, the neurons respond to various extracellular signals to extend their neurites and innervate their specific targets. At the point where two neurons communicate, there is a specialized structure called synapse. It can be formed on the spine or shaft of the dendrite, as well as the cell body or even between two axons. (b) Synaptic transmission at the central synapse. At the presynaptic terminus, depolarization leads to the opening of the voltage-dependent Ca<sup>2+</sup> channels. The resulting influx of Ca<sup>2+</sup> ions initiates the fusion of synaptic vesicles, which contain the neurotransmitter, to the pre-synaptic membrane, leading to the release of neurotransmitter to the synaptic cleft. Different neurotransmitter molecules are used by different populations of neurons in the CNS. Each type of the neurotransmitter molecules interact with the specific receptors at the postsynaptic membrane, which may be themselves ion channels, and result in a change in conformation of the receptors. This will allow opening of the ion channels and the influx of ions. Depending on the nature of the neurotransmitter receptors, the ions can be either positive (Na<sup>+</sup> or Ca<sup>2+</sup>) or negative (Cl<sup>-</sup>), leading to depolarization (excitatory) or hyperpolarization (inhibitory) of the postsynaptic neuron. A single postsynaptic neuron can receive and integrate many inputs, either excitatory or inhibitory, from numerous neurons. (c) Synaptic transmission at the NMJ. The NMJ consists of a presynaptic motor neuron, a postsynaptic muscle fiber, and the terminal Schwann cell. A junctional cleft, which contains a layer of extracellular matrix called the basal lamina, separates the pre- and postsynaptic components of the NMJ. The postsynaptic membrane on the muscle fibers is organized into many invaginations called the junctional folds, which are opposite to the active zone, where the neurotransmitter acetylcholine is released. Unlike the diverse arrays of neurotransmitter molecules at the central synapse, acetylcholine is the only neurotransmitter utilized at the mammalian NMJ. In addition, most muscle fibers are innervated by only one motor neuron in the mature animal, while the postsynaptic neuron in the CNS can receive hundreds of inputs from different neurons.

the three subtypes of ligand-gated ion channel (ionotropic receptor) for the neurotransmitter glutamate, and is the major receptor responsible for the excitatory synaptic transmission in the CNS. Because the PKA-induced phosphorylation of GluR1 is important for the synaptic incorporation of AMPA receptors in hippocampal neurons [9], it is plausible that Cdk5 is involved in the modulation of synaptic transmission through the phosphorylation of DARPP32 and the subsequent regulation of PKA activity. Furthermore, treating dissociated neurons from the striatum with roscovitine increases the whole-cell voltage-gated Ca<sup>2+</sup> current in a DARPP-32-dependent manner [8].

Voltage-gated Ca<sup>2+</sup> channels are involved in diverse functions in the pre- and post-synaptic transmission, such as regulation of neurotransmitter release, modulation of synaptic transmission and synaptic plasticity (reviewed by Ref. [10]). As the voltage-gated Ca<sup>2+</sup> channels can be regulated by PKA phosphorylation [11], they could be yet another potential target of the Cdk5/DARPP32/PKA

cascade in the regulation of synaptic transmission in the CNS. Additionally, the Cdk5-mediated DARPP-32 phosphorylation is stimulated by the neurotransmitter glutamate via the metabotropic glutamate receptor (mGluR), which is not an ion-channel itself but a G protein-coupled receptor that exerts its effect via the formation of secondary messenger. Stimulation of mGluR by the agonist DHPG increases Cdk5 activity via activation of casein kinase 1, which then leads to phosphorylation of DARPP-32 at threonine 75 [12].

Cdk5 also regulates synaptic transmission by inducing phosphorylation of the NR2A subunit of NMDA receptors at serine 1232 [5]. NMDA receptor represents yet another subtype of ionotropic glutamate receptor, which plays important roles in neuronal plasticity. Cdk5 associates with NR2A and PSD-95 in the brain, and phosphorylation of NR2A is reduced in *cdk5*<sup>-/-</sup> mice. In addition, the induction of long-term potentiation (LTP) in the CA1 region of the hippocampus, which depends on NMDA receptors, is abolished upon inhibition of Cdk5 by



**Fig. 2** Signal transduction of Cdk5 at the central synapse and the neuromuscular junction (NMJ). (a) Cdk5 regulates neurotransmitter release at the presynaptic terminus (upper) of the central synapse via phosphorylation of voltage-dependent calcium channel (VDCC). On the postsynaptic side, activation of metabotropic glutamate receptor (mGluR) enhances Cdk5 activity, which leads to phosphorylation of DARPP32, subsequent inhibition of PKA, and reduced phosphorylation of the GluR1 subunit of the AMPA receptor. The Cdk5/DARPP32/PKA cascade also regulates VDCC at the pre- and/or postsynaptic neurons. Cdk5 also phosphorylates the NR2A subunit of the NMDA receptor, while the calcium influx via the NMDA receptor regulates the interaction between p35 and CaMKII. (b) Cdk5 is activated by neuregulin (NRG) at the NMJ and, in turn, activates the NRG receptor ErbB via serine phosphorylation. The induction of Cdk5 activity is required for the NRG-induced AChR transcription at the subsynaptic nuclei. The MAP kinases ERK and JNK are also involved in mediating the NRG-induced AChR transcription via the Ets-related transcription factor GABP (reviewed by Ref. [65]). Dotted arrow represents proposed mechanism.

roscovitine [5]. The Cdk5 activators p35 and p39 interact with  $\alpha$ -actinin and the  $\alpha$ -subunit of calmodulin-dependent protein kinase II (CaMKII) at the synapse, and their interaction is enhanced by glutamate via stimulation of the NMDA receptor [13]. Although the functional consequence of the association between Cdk5 and CaMKII is not clear, it raises an interesting possibility of a cross-talk between Cdk5 and CaMKII in modulating synaptic transmission and neuronal plasticity.

### Cdk5 and p35 at the NMJ

Although Cdk5 activity was initially found to be most prominent in the brain, both Cdk5 and its activator p35 are also highly expressed in embryonic muscle. In particular, both proteins become localized at the NMJ during early postnatal stages, suggesting a potential role for p35/Cdk5 in NMJ formation and maintenance [14]. Neuregulin (NRG), a nerve-derived factor that induces the synapse-specific transcription of nicotinic acetylcholine receptor (AChR) at the NMJ (reviewed in Ref. [15]), increases both the expression of p35 and the activity of Cdk5 in the muscle cell line C2C12 [14]. Moreover, the NRG receptor ErbB associates with the p35/Cdk5 complex in muscle and is

activated by Cdk5 via serine phosphorylation, indicating that Cdk5 can be a component and potential downstream target of the NRG signaling pathway (Fig. 2b). Consistent with this hypothesis, inhibition of Cdk5 by roscovitine, or by a dominant-negative Cdk5 construct, or antisense oligonucleotides attenuates NRG-induced ErbB activity. The subsequent phosphorylation of the MAP kinase ERK and the induction of AChR  $\alpha$  and  $\epsilon$  subunits expression are also reduced. Strikingly, overexpression of the activator p35 alone, either by transfection into C2C12 muscle cells or injection into tibialis anterior muscle, can increase the AChR promoter activity without the addition of NRG. Together, these data strongly suggest a novel role for Cdk5 as a crucial downstream mediator of the NRG-induced AChR gene expression in muscle [14].

Does Cdk5 activity regulate the activity of other proteins at the postsynaptic side of the NMJ? Interactions between p35 and several nuclear proteins in muscle have been identified by using yeast two-hybrid screening, raising a possible interesting link between Cdk5 and gene transcription (Z. Li and N.Y. Ip, unpublished). Moreover, the Cdk-related kinase Pctaire1 has also been identified as a novel downstream target of Cdk5 in

muscle [16]. Pctaire1 interacts directly with p35 in muscle. Similar to Cdk5, Pctaire1 is also localized at the NMJ. The kinase activity of Pctaire1 is increased by Cdk5 *in vitro* through phosphorylation on serine 95, and Pctaire1 activity in muscle is significantly reduced in Cdk5-null mice, confirming that Pctaire1 is a downstream substrate of Cdk5 in muscle.

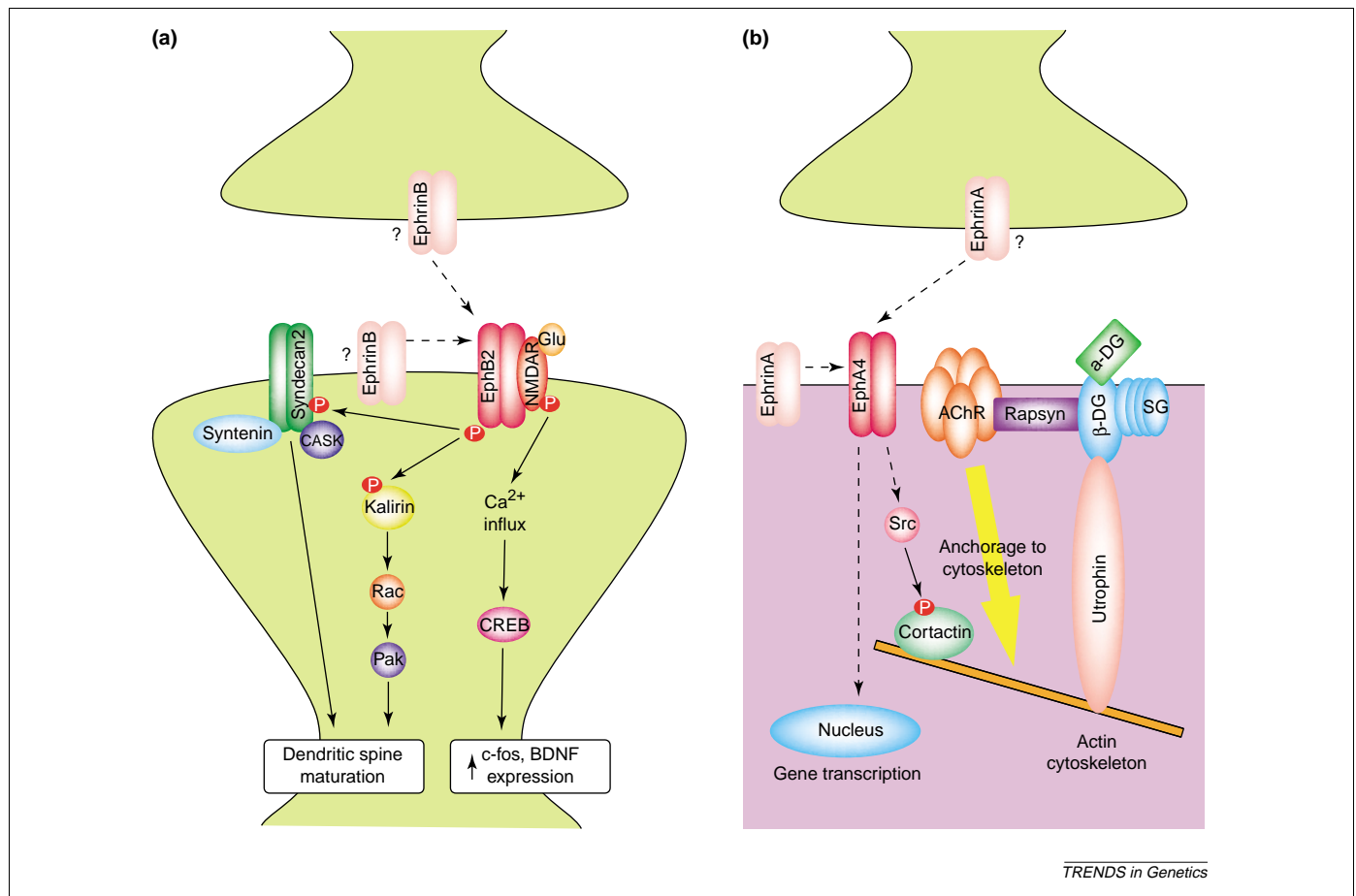
Recently, Pctaire1 was demonstrated to regulate neurite extension in neuroblastoma, suggesting a possible link between Pctaire1 and cytoskeleton [17]. Considering this finding, it appears attractive to investigate whether the p35–Pctaire1 interaction in muscle might regulate the clustering of postsynaptic proteins via effects on the cytoskeleton.

Finally, the functions of Cdk5 in NMJ development do not appear to be restricted to the postsynaptic muscle side, as analysis of Cdk5 null mice reveals defects in intramuscular neuritic projections and branching pattern (A.K.Y. Fu and N.Y. Ip, unpublished). In addition, the Cdk5 and p35-associated kinase activity in muscle increases prominently after nerve denervation, suggesting that Cdk5 and

p35 could also have important physiological roles in muscle after nerve injury [18].

### Ephrins and Eph receptors: functions at the synapses and the NMJ

Although Eph receptors were initially identified as major repulsive signaling molecules in axon guidance and topographic mapping during nervous system development, emerging evidence suggests that they have additional roles in synapse formation and synaptic plasticity (Fig. 3a). The family of Eph receptors has fourteen mammalian members, and they are activated by membrane-bound ligands called ephrins. Eight mammalian ephrins have been identified, which are classified into A and B subclasses on the basis of their modes of membrane anchorage. The ephrin-A ligands (ephrin-A1 to A5) are GPI-anchored, whereas B class ephrins (ephrin-B1 to B3) contain a transmembrane domain. In general, the A class Eph receptors are preferentially activated by ephrin-A ligands, and the EphB receptors are activated by the ephrin-B ligands. Promiscuous binding and functional



**Fig. 3.** Signaling pathways of Eph receptors at the central synapse and the NMJ. (a) At the central synapse, activation of EphB2 induces tyrosine phosphorylation and clustering of syndecan-2, which is associated with cytoplasmic ligands such as syntenin and CASK. EphB2 activation also induces tyrosine phosphorylation of the GDP/GTP exchange factor kalirin, which leads to activation of Rac and Pak. Both pathways result in maturation of dendritic spines. Activation of EphB2 also phosphorylates the NR2B subunit of the NMDA receptor, leading to increased  $\text{Ca}^{2+}$  influx, enhanced phosphorylation of CREB and increased transcription of c-fos and BDNF. It is not clear whether the ephrinB ligands are localized at the pre- or postsynaptic terminus (or both). (b) Activation of EphA4 induces tyrosine phosphorylation of the actin-binding protein cortactin in muscle, which may regulate the dynamics of actin cytoskeleton and, in turn, affect the stability of postsynaptic proteins at the NMJ.  $\alpha$ -DG,  $\beta$ -DG and SG represent  $\alpha$ - $\beta$ -dystroglycan and sarcoglycan, respectively. Together with utrophin, the dystroglycans and sarcoglycans form the utrophin-associated protein complex at the NMJ. Through the 43 kD protein rapsyn, the utrophin-associated protein complex links AChR to the actin cytoskeleton and may represent yet another potential target for EphA4 in the maintenance of NMJ. Another possible function of EphA4 in muscle may involve regulation of gene transcription. Dotted arrows represent proposed mechanisms. Whether ephrin-A ligands are concentrated at the presynaptic motor neuron is not clear.

redundancy has been observed among different members of the same family, although there are wide variations in terms of binding affinities (reviewed by Ref. [3]).

One characteristic feature of ephrins is that they are membrane-bound ligands, and the membrane anchorage is essential for their activation of the receptors [19]. Thus, to study the functions of ephrins *in vitro*, the ligands have to be fused with a dimeric Fc tag and then artificially clustered by adding anti-Fc antibody. Another unusual feature of ephrins is that the ligands can themselves trigger reverse signaling upon interaction with the receptors [20–22]. Thus, the ligand-expressing cells transduce intracellular signaling and elicit biological response when they interact with cells expressing the receptors.

### Eph receptors and central synapse

Eph receptors and the transmembrane ephrin-B ligands are localized at the synapses of cultured hippocampal neurons, and the localization might be mediated by interactions with the PDZ domains of PICK1 and GRIP, the proteins that interact with AMPA receptors [23,24]. Stimulation by ephrin-B1 or overexpression of wild-type EphB2 in cultured cortical and hippocampal neurons increases the number of postsynaptic specializations, and introduction of dominant-negative EphB2 reduces the density of postsynaptic NMDA receptors, indicating that B class ephrins might be responsible for the initial clustering of neurotransmitter receptors in the CNS [25].

Recent studies also provide evidence for functional roles of Eph receptors in the formation and maturation of dendritic spines, the principal postsynaptic targets for excitatory synapses, in the hippocampus during development. One study showed that the activation of EphB2 leads to tyrosine phosphorylation of syndecan-2, a heparan sulfate proteoglycan that has been implicated in dendritic spine formation [26]. EphB2 is associated and co-localized with syndecan-2 at the dendritic spines, and introduction of kinase-inactive EphB2 blocks the dendritic spine formation induced by syndecan-2 in cultured hippocampal neurons. Another study demonstrated that EphB2 can also regulate spine morphogenesis through the GDP/GTP exchange factor kalirin. Ephrin-B1 induces tyrosine phosphorylation and synaptic clustering of kalirin, and the ephrin-B1-induced spine formation is inhibited by introduction of an inactive kalirin mutant [27]. Notably, as a kalirin inactive mutant inhibits the ephrin-B1-induced phosphorylation of Pak, Rac and Pak turn out to be key downstream targets of kalirin in the regulation of EphB2-mediated dendritic spine morphogenesis [27].

Ephrins also modulate synaptic plasticity in the mature nervous system. Perfusion with dimeric ephrin-A5 induces an LTP-like effect in hippocampal slices, and injection of dimeric ephrin-A5 into the hippocampus enhances learning behavior in mice [28,29]. Furthermore, homozygous mice lacking EphB2 have impaired long-lasting LTP [30,31]. The modulation of neuronal plasticity by ephrin-B might involve regulation of NMDA receptor function. EphB2 interacts directly with the NR1 subunit of the NMDA receptor, and the interaction is enhanced upon ephrin treatment [25]. Ephrin-B2 also induces tyrosine

phosphorylation of NR2B in cultured cortical neurons by the Src family kinases and enhances glutamate-induced  $\text{Ca}^{2+}$  influx, leading to increased activity of the transcriptional regulator CREB and enhanced expression of c-fos and BDNF [32]. In addition, Eph receptors are involved in the maintenance of postsynaptic morphology in the adult hippocampus, which could be yet another mechanism to regulate neuronal plasticity. Ephrin-A3, for example, is expressed in the astrocytic processes near the postsynaptic spines, and treatment of adult hippocampal slices with ephrin-A3 leads to retraction of dendritic spines. Hippocampal neurons from EphA4 knockout mice exhibit disorganized spine morphology and no longer respond to ephrin-A3, indicating that EphA4 is the dominant Eph receptor that mediates the effect of ephrin-A3 in remodeling dendritic spine morphology [33].

### What roles do Eph receptors and their corresponding ephrin ligands have at the NMJ?

Although many members of the Eph family were originally identified in the CNS, Eph receptors are also expressed in other tissues, including muscle. Particularly, two members of the Eph receptor family, EphA4 and EphA7, and their cognate ligand ephrin-A2, are localized at the postsynaptic apparatus of the adult NMJ [34]. Although more abundant in muscle during early developmental stages, the synaptic localization of both Eph receptors and ephrin-A2 is observed at late postnatal stages, during which the stability of AChR is higher than that of newborn rats. It is therefore tempting to speculate that ephrins might affect the stability of the postsynaptic apparatus of the NMJ. In this regard, it is noteworthy that EphA4 co-immunoprecipitates with the actin-binding protein cortactin in muscle, and cortactin is tyrosine phosphorylated upon activation of EphA4 [34]. Cortactin can promote the cross-linking of actin filaments, and tyrosine phosphorylation of cortactin regulates remodeling of the actin cytoskeleton [35]. Actin, on the other hand, is crucial for the stability of AChR clusters in cultured muscle cells. Interestingly, actin polymerization and cortactin localization accompany agrin-induced AChR clusters formation in cultured myotubes [36], which prompts the hypothesis that EphA4 signaling at the NMJ could be important to maintain the postsynaptic specialization through effects on the actin cytoskeleton (Fig. 3b). Alternatively, given the precedence at the central synapse, ephrins might regulate gene transcription in muscle that, in turn, modulates synaptic functions at the NMJ. Indeed, the signal transducer and activator of transcription (Stat) is tyrosine phosphorylated by ephrin-A in muscle (K.O. Lai and N.Y. Ip, unpublished).

### Agrin is central in the formation of the NMJ, neuronal synaptogenesis and neurite outgrowth

The significance of agrin and its putative receptor MuSK in the formation of the postsynaptic specialization has been well documented (reviewed in Ref. [15]). The postsynaptic specialization is formed in the middle of the muscle, near the entry of the motor neuron. Originally, it was thought that the motor axon induces AChR clusters at a site where it first makes contact with the muscle, with

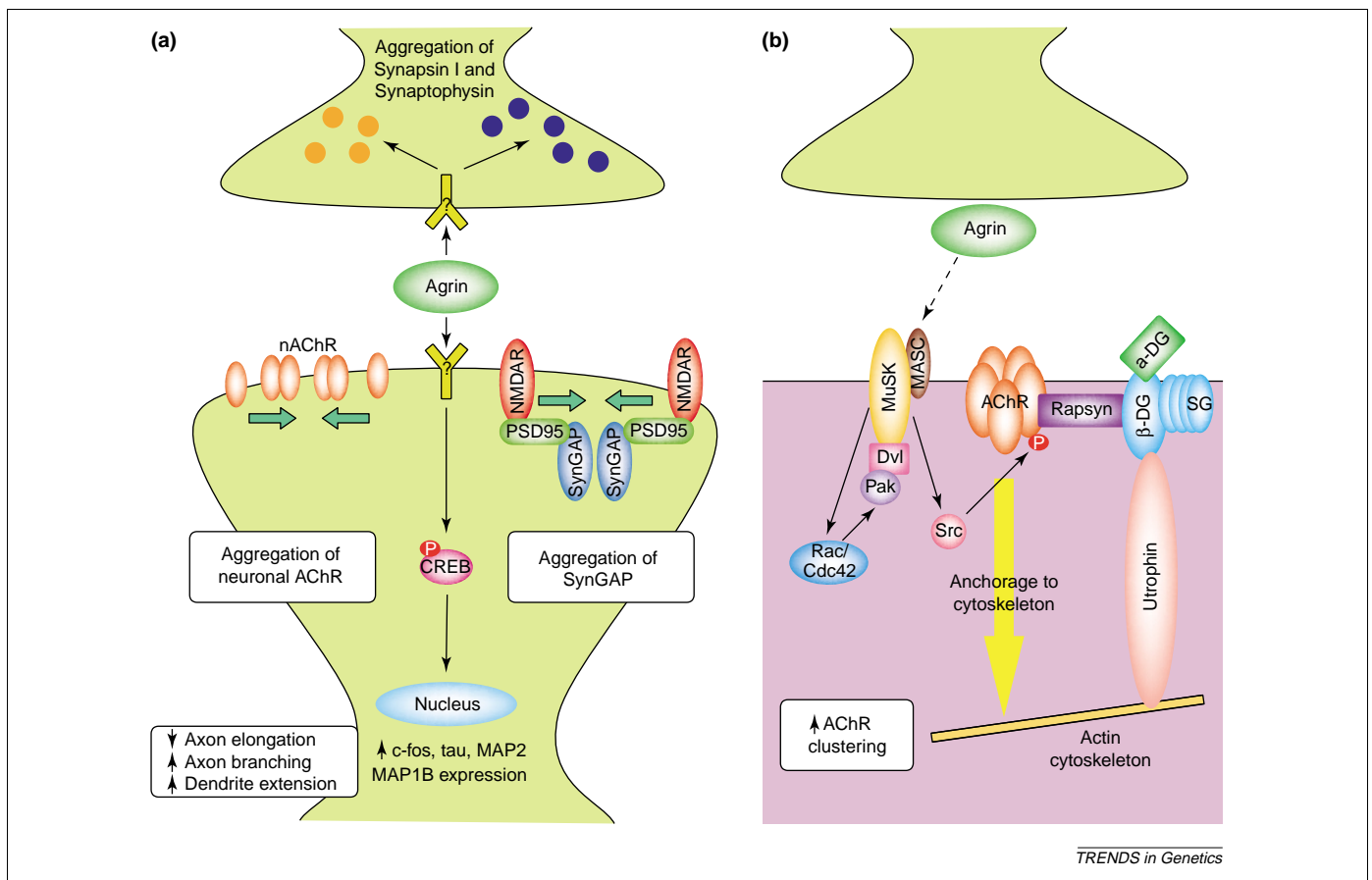
the site being random rather than predisposed on the muscle fibers. However, recent evidence suggests that the patterning mechanism is a cell-autonomous feature of the muscle. In knockout mice that contain null mutation of topoisomerase II $\beta$  or HB9, the motor axon fails to innervate the muscle, yet a central band of AChR clusters is detected, indicating that the developing muscle is pre-patterned [37–39]. These aneural AChR clusters also exist in homozygous mice lacking neural agrin [39], suggesting that agrin is not involved in the initiation of the postsynaptic specializations.

However, agrin is important in the maintenance of the AChR clusters formed initially, because the number and size of AChR clusters decrease in *agrin*<sup>-/-</sup> mice [38]. Agrin acts on the receptor tyrosine kinase MuSK (Fig. 4b), the expression of which in mammals appears to be restricted to muscle [40]. Agrin induces rapid tyrosine phosphorylation of MuSK, which is essential for the agrin-induced phosphorylation of AChR $\beta$  subunits [41,42]. Agrin cannot bind directly to purified MuSK or MuSK expressed in fibroblasts, suggesting the existence of a co-receptor (called MASC) specifically expressed in muscle cells [41]. Homozygous knockout mice with a targeted deletion of MuSK have a more severe phenotype than the *agrin*<sup>-/-</sup> mice, confirming the significance of MuSK in AChR clustering *in vivo* [43]. It should be noted that the aneural

AChR clusters are absent in muscle of mice lacking MuSK but not agrin [38,39]. In addition, exogenous expression of MuSK in Cos7 cells leads to autophosphorylation of the receptor, and the aggregation of AChR is elevated in *Xenopus* embryos injected with MuSK [44]. These observations therefore raise the intriguing possibility that ligand-independent activation of MuSK might exist, initiating the clustering of AChR on the muscle fiber independently of innervation.

The signaling pathways leading to the formation and maintenance of AChR clusters have begun to be elucidated. Agrin activates the Src family of tyrosine kinases in cultured myotubes and, in turn, phosphorylate the  $\beta$ -subunit of the AChR, which is important in the formation and maintenance of AChR clusters [45,46]. Rac, Cdc42 and Pak are also activated in C2C12 muscle cells upon agrin treatment [47,48], and dominant-negative mutants of Rac or Cdc42 prevent the formation of AChR clusters. Moreover, constitutively active Rac triggers AChR aggregates formation without agrin treatment, indicating that activation of Rac is sufficient for AChR clustering [47].

Although the function of agrin is best studied at the NMJ, its expression is not restricted to the motor neuron but is widespread throughout the CNS. Agrin expression is regulated by neural activity (reviewed in Ref. [49]), raising



**Fig. 4.** Signal transduction of agrin in the neuron and the muscle cell. (a) Agrin is involved in clustering synapsin I and synaptophysin at the presynaptic terminus, as well as neuronal AChR and synGAP on the postsynaptic sympathetic and hippocampal neurons, respectively. Agrin also regulates gene expression of cytoskeletal proteins in the neuron and, in turn, affects neurite extension. The identity of the neuronal receptor of agrin that mediates these responses remains to be confirmed. (b) At the NMJ, agrin activates MuSK, which is associated with the hypothetical receptor MASC in muscle. Agrin signaling triggers activation of Rac, Pak, and Src, which are required for the clustering of AChR. Pak is linked to MuSK via the multi-domain protein Dishevelled (Dvl) [48].

the possibility that agrin might participate in the formation of neuron–neuron synapses. Treatment of cultured hippocampal neurons with agrin induces serine phosphorylation of the transcriptional regulator CREB and expression of the immediate early gene *c-fos* [50,51]. However, cortical and hippocampal neurons derived from mice lacking the *z+* agrin, which is much more potent than the *z-* isoform in clustering AChR in myotubes, exhibit normal synapse formation. No significant difference in the clustering of pre- and postsynaptic proteins and synaptic transmission was observed between agrin-deficient mice and wild-type or heterozygote litter mates [52,53]. In contrast, synaptogenesis is disrupted when cultured hippocampal neurons are treated with antisense agrin [54,55]. The punctated staining of synapsin I and synGAP is reduced in hippocampal neurons treated with antisense agrin, which can be reversed by addition of *z+*, but not *z-* recombinant agrin.

Antisense agrin also affects the synaptic transmission and rate of spontaneous exocytosis in hippocampal neurons [55]. The contrasting interpretations on the significance of agrin in neuronal synaptogenesis could be explained by functional redundancy and compensatory mechanisms in agrin-deficient mice. A recent study has revealed defects in the aggregation of synaptophysin and neuronal AChR in sympathetic neurons derived from agrin-deficient mice, thereby supporting a role of agrin in synaptogenesis of neurons [56]. The mechanisms underlying agrin-induced synaptogenesis, however, remain to be determined (Fig. 4a). In particular, the neuronal agrin receptor has yet to be identified. Although MuSK expression seems to be restricted to muscle in mammals, significant expression of MuSK is detected in the CNS of *Xenopus* and chicken, raising the possibility that MuSK is responsible for mediating agrin-induced synaptogenesis in the CNS of lower vertebrates [57,58].

An earlier study showed that neurite extension of chick ciliary ganglion was inhibited when co-cultured with agrin-expressing CHO cells. In addition, both the *z+* and *z-* isoforms of agrin are able to inhibit neurite outgrowth, suggesting a possible role of the muscle-derived *z-* isoform of agrin in regulating the growth of the presynaptic motor neuron [59]. Agrin can also regulate neurite extension in CNS neurons (Fig. 4a). Reduction of axonal length and increase in axonal branching and dendritic extension of hippocampal neurons is observed in the presence of recombinant agrin. In contrast, adding antisense agrin to hippocampal neurons results in longer axon and shorter dendrites. The effect of agrin on neurite extension might involve the modulation of cytoskeleton composition, because the expression of tau and the microtubule-associated proteins MAP1B, MAP2 is induced by agrin [60]. Lastly, addition of agrin to *Xenopus* neuron–muscle culture or injection of MuSK into *Xenopus* embryos regulates neurite extension of motor neurons (Z. Xu *et al.*, unpublished), suggesting that the effect of agrin on neurite outgrowth in *Xenopus* could be mediated by MuSK.

## Conclusion

Enormous progress in the past few years has unraveled the multiple functions of signaling molecules at the growth

cone, central synapse, and NMJ. We have discussed the potential significance of Cdk5, Eph receptors, agrin and MuSK in the diverse aspects of nervous system development and neuronal function, addressing the regulation of neurite outgrowth, axon guidance, synaptogenesis, synaptic plasticity, and NMJ formation and maintenance. Given that key molecules of axon guidance are now also implicated in synaptic function, it might only be a matter of time before additional axon guidance molecules will be shown to have similar extensions of their function. The semaphorin family could be one such example. Recent studies identified the interaction of semaphorin 4F with PSD-95 at neuronal synapses, and semaphorin 1a has been implicated in synapse formation in *Drosophila* [61,62]. Because many molecules crucial for synaptic transmission and plasticity at the neuronal synapses are also localized at the postsynaptic apparatus of the NMJ, it remains to be determined whether the function of semaphorin also extends to the NMJ. Conversely, signaling molecules crucial for NMJ formation might yet have undiscovered synaptic functions in the CNS. In this context, it is worthwhile mentioning neuregulin, which was recently demonstrated to modulate synaptic transmission of GABAergic neurons of the hippocampus [63]. Moreover, the NRG receptor ErbB4 interacts with PSD95 at neuronal synapses, and activation of ErbB4 regulates LTP in the CA1 area of the hippocampus [64]. It will be interesting to explore whether neuregulin also has a possible function in neurite extension during early development, consistent with the molecular overlap discussed here between synaptogenesis and neurite outgrowth.

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