Synaptic Plasticity: One STEP at a time

Steven P. Braithwaite[1], S. Paul[2] A.C. Nairn[3] and P.J. Lombroso[4]

[1] AGY Therapeutics Inc, 270 E. Grand Avenue, South San Francisco, CA 94080

[2] Dept of Neurology, University of New Mexico, Albuquerque, NM 87131

[3] Dept of Psychiatry, Yale University School of Medicine, New Haven, CT 06520

[4] Child Study Center, Yale University School of Medicine, New Haven, CT 06520

Abstract

STriatal Enriched tyrosine Phosphatase (STEP) has recently been identified as a critical player in the regulation of synaptic function. It is highly restricted to neurons within the CNS and acts by down-regulating the activity of the MAP kinases, the tyrosine kinase Fyn, and NMDA receptors. By modulating these substrates, STEP acts on several parallel pathways that impact upon the progression of synaptic plasticity. Recent advances have demonstrated the importance of STEP in normal cognitive function and its possible involvement in cognitive disorders, such as Alzheimer's disease.

Introduction

Tyrosine phosphorylation of synaptic receptors and signaling molecules regulates synaptic activity (Soderling and Derkach, 2000; Salter and Kalia, 2004). Considerable work has characterized the kinases involved in activity-dependent synaptic plasticity, with relatively less emphasis on the participating protein tyrosine phosphatases (PTPs). The identification and characterization of tyrosine phosphatases that participate in this process has begun and a number of PTPs specifically expressed within the brain have been identified (Paul and Lombroso, 2003).

One of these PTPs was named STEP (for STriatal-Enriched tyrosine Phosphatase, also known as PTPN5), and recent evidence suggests it plays an important role in synaptic plasticity. The past decade has seen considerable advances in our understanding of the function of STEP, as well as the identification of several target proteins by which STEP controls the development of synaptic plasticity. This review concentrates on three groups of proteins that STEP regulates: the mitogen-activated protein kinases (MAPKs), the tyrosine kinase Fyn, and the NMDA receptor complex. Tyrosine phosphorylation of one member of the MAPK family, the extracellular signal regulated kinase (ERK), is necessary for the expression and maintenance of synaptic plasticity in many brain regions (Sweatt, 2004), and disruption of the ERK pathway leads to a disruption of learning and memory. Activation of the Src family of non-receptor tyrosine kinases is also regulated by tyrosine phosphorylation. One of the functions of these kinases is to phosphorylate NMDA receptors, thereby modulating their channel conductance properties and facilitating their movement to neuronal plasma membranes (Salter and Kalia, 2004). This potentiates their activity and is required for the induction of several forms of long-term potentiation (LTP) and long-term depression (LTD) (Nicoll, 2003; Collingridge et al., 2004). This review discusses the properties of STEP that are necessary for its ability to regulate these three families of proteins and its role in synaptic function, learning and CNS pathology.

Molecular properties of STEP

STEP is specifically expressed within neurons of the central nervous system (Lombroso et al., 1991). As its name indicates, the highest expression level is within the striatum (Lombroso et al., 1993). However, more recent work has found that it is expressed at lower levels in multiple brain regions including the neocortex, amygdala, hippocampus, and embryonic spinal cord (Boulanger et al., 1995; Pelkey et al., 2002).

Tyrosine phosphatases are broadly divided into the receptor-like and the non-receptor, intracellular phosphatases (Stoker, 2005; Paul and Lombroso, 2003). Of the approximately 100 tyrosine phosphatases identified in the human genome, STEP falls into a small subset of the non-receptor tyrosine phosphatases (Andersen et al., 2004; Alonso et al., 2004). Based on sequence homology, its closest relatives are HePTP and PTP-SL that are also expressed in a restricted fashion, with HePTP found only in leucocytes and PTP-SL enriched within the cerebellum (Zanke et al., 1992; Sharma and Lombroso, 1995; Hendricks et al., 1995; Chirivi et al., 2004).

STEP mRNA is alternatively spliced into two main variants (Figure 1). The protein products are termed $STEP_{46}$ and $STEP_{61}$ based on their observed electrophoretic mobility (Lombroso et al., 1991; Bult et al., 1996). $STEP_{46}$ is cytosolic, while $STEP_{61}$ is membrane-bound and differs from $STEP_{46}$ by the presence of an extra 172 amino acids at its N-terminus (Figure 1). This N-terminal sequence targets $STEP_{61}$ to intracellular organelles including the endoplasmic reticulum and the postsynaptic density (Boulanger et al., 1995; Oyama et al., 1995, Bult et al., 1996). Additional alternatively spliced variants are expressed that lack an active phosphatase domain (Bult et al., 1997; Sharma et al., 1995). The functions of these inactive variants are not known, although it is interesting to note that alternatively spliced variants exist for a number of receptor tyrosine kinases that bind to their substrates and preserve the phosphorylation levels of these substrates (Baxter et al., 1997).

Both $STEP_{46}$ and $STEP_{61}$ have a C-terminal domain of approximately 280 amino acids that contains a catalytic site with the consensus sequence (I/V)HCXAGXXR(S/T)G (Figure 1). A kinase-interaction motif (KIM) is located N-terminal to the phosphatase domain. This domain is uniquely found in STEP, HePTP and PTP-SL and is the binding site for members of the MAP kinase family. The N-terminal domain of $STEP_{61}$ also contains two polyproline rich regions that may be important in mediating, at least in part, its interactions with substrates (for example, Fyn; Nguyen et al., 2003). Two hydrophobic transmembrane domains and two PEST sequences are also present in $STEP_{61}$. The latter sequences are potential sites for proteolytic cleavage, and two studies have shown that $STEP_{61}$ is cleaved after hypoxia/anoxia in rat forebrain or excitotoxic glutamate stimulation (Gurd et al., 1999; Nguyen et al., 1999); however, whether the PEST sites in $STEP_{61}$ are the points of cleavage remains to be determined.

Baseline expression of STEP isoforms varies depending on the tissue examined. Thus, the striatum and portions of the amygdala (central nucleus) express both $STEP_{46}$ and $STEP_{61}$. The hippocampus, neocortex, spinal cord, and lateral aspects of the amygdala only express the larger $STEP_{61}$ variant. This variation in isoform levels is reflected in the stronger immunohistochemical staining within the striatum and central nucleus compared, for example, to the hippocampus. STEP is expressed throughout the length of the neuron in a Golgi-like impregnation pattern (Boulanger et al., 1995). Thus, the somata, dendritic arbors and axonal processes are STEP immunoreactive. The projection targets of striatal neurons (globus pallidus and substantia nigra) have only neuritic staining with no detectable STEP immunoreactivity in cell bodies. A conclusion of this latter finding is that, although the majority of work to date has emphasized the function of STEP postsynaptically, STEP is also present presynaptically and is likely to regulate synaptic transmission through presynaptic mechanisms.

Regulation of STEP activity

The regulation of STEP activity has focused upon the striatum where it is expressed in medium spiny neurons that make up about 90% of the neuronal cell types within this brain region (reviewed in Packard and Knowlton, 2002). Dopaminergic inputs from the midbrain and glutamatergic afferents from the cortex converge on the spines of these neurons (Kotter, 1994). Considerable evidence indicates that the integration of these two synaptic inputs promotes their impact on synaptic function and plasticity, although the mechanisms for this remain unclear (Cepeda and Levine, 1998). Recent findings suggest that STEP is involved in the integration of these signals.

Stimulation of dopamine D1 receptors is coupled to adenylyl cyclase through $G\alpha_s$ leading to increased cAMP levels, which in turn activates the PKA pathway. PKA phosphorylates both $STEP_{46}$ and $STEP_{61}$ at a regulatory serine residue within their respective KIM domains, as well as a serine residue in the novel 172 amino acid N-terminal domain of $STEP_{61}$. The effect of phosphorylation is to decrease STEP's enzymatic activity (Figure 2). Phosphorylation within the KIM domain prevents STEP from binding to ERK; however, it remains to be determined whether other substrates of STEP are also affected by phosphorylation within the KIM domain.

Phosphorylation of STEP is stimulated by D1 selective agonists, blocked by D1 receptor antagonists and not blocked by D2 receptor antagonists (Paul et al., 2000). Glutamate stimulation reverses this process and activates STEP. Stimulation of NMDA receptors, but not AMPA receptors, results in the influx of Ca^{2+} and activation of the serine/threonine phosphatase calcineurin. As a result, STEP is dephosphorylated at the KIM domain regulatory serine residue (Paul et al., 2003). Furthermore, it has been demonstrated that PP-1 can act to dephosphorylate the regulatory serine residue in the KIM domain of STEP (Valjent et al, 2005) and the highly related HePTP (Nika et al., 2004).

STEP functions

The specificity of PTPs towards their substrates arises through amino acid modules that target PTPs to cellular compartments, while additional motifs lead to their interactions with substrate proteins. As mentioned above, STEP, along with its closest relatives HePTP and PTP-SL, contain a KIM domain that is necessary for binding to MAPK family members ERK, p38α, and JNK (Pulido et al., 1998). All three of these PTPs dephosphorylate the regulatory tyrosine in the activation loop of MAPKs and thereby inactivate them (Zuniga et al., 1999; Saxena et al., 1999; Pulido et al., 1998; Paul et al., 2003).

The ability of STEP to regulate ERK (Figure 3) has been shown in a number of studies. In corticostriatal cultures, ERK is rapidly activated (within 2 minutes) in response to glutamate stimulation, followed by a delayed inactivation of ERK to baseline phosphorylation levels by 20-30 minutes. This delayed inactivation of ERK is mediated by STEP through its delayed dephosphorylation within the KIM domain in response to NMDA receptor-dependent activation of calcineurin (Paul et al., 2003) (Figure 2). Thus, STEP acts to regulate the temporal profile of ERK activity, and consequently helps to control its translocation to the nucleus, and subsequent downstream nuclear signaling.

In a second study, STEP was found to play an important role in a signal transduction cascade that mediates the effects of psychostimulant drugs on ERK activation (Valjent et al., 2005). Psychostimulant drugs of abuse exert their addictive effects by increasing extracellular dopamine in the nucleus accumbens, where they likely alter the plasticity of corticostriatal glutamatergic transmission. Cocaine and amphetamine activate ERK in a subset of medium spiny neurons of the dorsal striatum and nucleus accumbens, through the combined action of NMDA and D1-dopaminergic receptors. The activation of ERK involves D1-dopamine receptor-dependent regulation of PKA, phosphorylation of the regulatory protein DARPP-32, inhibition of the serine/threonine phosphatase, PP-1, and inhibition of STEP. Thus, activation of ERK, by a

protein phosphatase cascade, functions as a detector of coincidence of dopamine and glutamate signals converging on accumbens medium spiny neurons and is critical for long-lasting effects of drugs of abuse.

Recently, a series of *in vivo* investigations directly tested the hypothesis that STEP might be involved in regulating synaptic plasticity (Paul et al., in press). ERK activation is required for the consolidation of many forms of long-term memory, including fear conditioning (Schafe et al., 2000). A substrate-trapping mutant of STEP was made cell permeable by attaching a TAT-peptide to the N-terminus. It was infused into the lateral amygdala to determine whether it would bind to ERK, disrupt ERK signaling, and thereby block consolidation of long-term memories after fear conditioning. Animals were trained on a standard protocol where a shock is paired with an acoustic cue. Short-term memory was not affected in these animals, implying that the substrate trapping TAT-STEP protein did not block the acquisition of this form of memory. However, 24 hours after fear conditioning, long-term memory was disrupted, indicating an effect on the consolidation of fear memories.

There were two striking observations in this study. The first was the rapidity of ERK activation after fear conditioning. Phosphorylated ERK (pERK) was detected in lateral amygdala neurons within five minutes of training, returned to baseline levels by 15 minutes, and then increased again by one hour. The initial activation of ERK is thought to occur through the convergence onto lateral amygdala neurons of auditory thalamic inputs in response to the conditioning stimulus (tone) and somatosensory thalamic inputs in response to the unconditioned stimulus (electrical foot shock). Both inputs are required for the establishment of LTP in the lateral amygdala and the consolidation of fear conditioning (Schafe and LeDoux, 2000; Blair et al., 2001).

Activation of ERK was followed within an additional few minutes by the *de novo* translation of STEP (Paul et al., in press). The translation of STEP was blocked by anisomysin,

not affected by actinomycin D, and blocked by inhibitors of MAPK. Importantly, neither shock alone nor tone alone led to ERK activation or STEP translation. Immediately after the *de novo* expression of STEP, pERK levels returned to baseline levels. These results support a feedback model by which STEP regulates the duration that ERK is active. Additional modulatory inputs are likely to be involved. For example, if a dopaminergic input arrives to these same neurons, then STEP will be phosphorylated and no longer interact with ERK, leading to a more persistent pERK signal. Additional studies are needed to determine whether the infused TAT-STEP that prevented the consolidation of fear conditioning did so through its ability to block ERK signaling only, or whether it also disrupts other components of synaptic plasticity, through the regulation of STEP substrates such as Fyn or NMDA receptors.

Mutations of PTPs in their catalytic domain create inactive variants that may be used as substrate-trapping proteins to identify potential substrates. Inactive PTPs bind to their substrates but do not release them, as release requires dephosphorylation of the target protein (Flint et al., 1997). This type of inactive STEP protein was used in the infusion experiments described above, and was also used to identify a second STEP substrate, the non-receptor tyrosine kinase Fyn (Figure 3) (Nguyen et al., 2002). STEP interacts with Fyn through its KIM domain, although a polyproline sequence present in $STEP_{61}$ is also involved in Fyn binding (Nguyen et al., 2002). Interestingly, the related tyrosine kinases, Src, Lyn and Pyk2, which are also present within the postsynaptic density, did not interact directly with STEP under the conditions used in this study (Nguyen et al., 2002). Two tyrosine residues are phosphorylated in the Src family of non-receptor kinases, and the enzymatic activity of these proteins depends upon which tyrosine is phosphorylated. STEP specifically catalyzes the dephosphorylation of Tyr^{420}, leading to the inactivation of Fyn. Conversely, a second PTP (PTPα) dephosphorylates Tyr^{531}, and phosphorylation of this residue activates Fyn (Zheng et al., 1992; Bhandari et al., 1998).

The NMDA receptor is a third potential STEP substrate. The NR1 subunit was initially shown to associate with STEP through co-immunoprecipitation experiments using hippocampal tissue (Pelkey et al., 2002) and more recently it has been shown that NMDA receptor subunits and STEP interact directly (Braithwaite et al, in press). STEP regulates NMDA receptor trafficking by controlling the level of tyrosine phosphorylation of the NR2B subunit (Snyder et al., 2005; Braithwaite et al, in press). Tyrosine phosphorylation of NR2B at Tyr^{1472} by Src-family members, including Fyn, is required for the movement of NMDA receptors into membranes (Dunah et al., 2004; Cheung and Gurd, 2001). Dephosphorylation of the NR2B subunit at that same residue leads to endocytosis of NMDA receptors through a clathrin- and adaptor protein-2-mediated mechanism (Lavezzari et al., 2003). Current studies are determining whether this is through the direct dephosphorylation of the NMDA receptor by STEP, an indirect effect through its ability to reduce Fyn activity and thus decrease NMDA Tyr^{1472} phosphorylation levels, or whether both mechanisms work together in a cooperative fashion (Figure 4).

An initial electrophysiological study looked at the ability of STEP to regulate NMDA receptor channel properties (Pelkey et al., 2002). STEP affects the function of synaptic NMDA receptors in both spinal cord cultures and hippocampal CA1 pyramidal neurons. Exogenously applied STEP decreased the open probability and mean channel open time of NMDA receptors in single channel recording from excised patches of spinal cord neurons (Pelkey et al., 2002). Furthermore, infusing a functionally inhibitory STEP antibody increased the NMDA receptor-mediated component of synaptic responses. Because NMDA receptors are critically important for the induction of LTP, it was important to examine the role of STEP in this form of synaptic plasticity (Pelkey et al., 2002). Microinfusion of active STEP protein into the postsynaptic neuron blocked LTP induction at hippocampal Schaffer collateral CA1 synapses. Conversely, infusion of the functionally inhibitory antibody caused an increase in basal synaptic transmission, thereby occluding LTP induction. Thus, STEP appears to directly affect the

conductance properties of NMDA receptors as well as regulating NMDA receptor trafficking, and together, these mechanisms oppose the development of synaptic plasticity.

Significance of STEP in pathological states

Recent studies have linked STEP to the cognitive decline observed in Alzheimer's disease (Snyder et al., 2005). The abnormal secretion of beta-amyloid peptide (Aβ) has been implicated in Alzheimer's disease, and the appearance of plaques and neurofibrillary tangles have been thought to be a pathogenic cause of the disorder. A second model posits that soluble Aβ interferes with synaptic function itself and at an earlier time point (Walsh et al., 2002; Kamenetz et al., 2003).

Snyder et al. (2005) directly tested the synaptic hypothesis of Alzheimer's disease by asking whether Aβ might disrupt NMDA receptor trafficking. Aβ promoted the endocytosis of NMDA receptors in hippocampal cultures without affecting the total level of these receptors. Moreover, a similar decreased level of glutamate receptors was found on neuronal plasma membranes in a mouse model of Alzheimer's disease that secretes high levels of Aβ. As mentioned above, exocytosis and endocytosis of NMDA receptors are regulated, in part, by tyrosine phosphorylation of the NR2B subunit. Aβ-induced endocytosis of NMDA receptors was blocked by preincubation of hippocampal cultures with the substrate-trapping TAT-STEP protein. The implication was that STEP might normally be involved in the endocytosis of glutamate receptors, and that it was being inappropriately activated by Aβ.

The study went on to determine the signaling pathway by which Aβ-induced endocytosis occurred (Figure 4). Aβ bound to the α7 nicotinic acetylcholine receptor (α7nAChR), leading to Ca^{2+} influx and activation of calcineurin. Calcineurin activity resulted in dephosphorylation of the regulatory serine within the KIM domain of STEP, thereby activating it. Active STEP could now dephosphorylate Fyn and/or NR2B, promoting endocytosis of NMDA receptors. A second

study has also implied a role for STEP in the actions of Aβ. In a different transgenic mouse model of Alzheimer's disease, the investigators found increased levels of α7 nAChR, decreased active Fyn, and increased STEP protein levels in the dentate gyrus (Chin et al., 2005).

It remains to be determined exactly how the substrate trapping TAT-STEP prevents NMDA receptor endocytosis. Given the fact that it acts as a substrate-binding protein, one possible model is that TAT-STEP binds to the Tyr^{1472} site and blocks normal dephosphorylation of that site. The increased tyrosine phosphorylation of NR2B would promote its localization at the plasma membrane. These observations support the hypothesis that one of the earliest pathological events in Alzheimer's disease is a tyrosine dephosphorylation-mediated endocytosis of glutamate receptors, and that this process may be involved in the progressive cognitive loss in affected patients. Because STEP is an integral part of the signaling pathway between Aβ and the NMDA receptor, inhibiting STEP activity is a potential avenue for new therapeutic agents in the treatment of Alzheimer's disease.

Conclusion

STEP regulates the activity of the MAPKs, Fyn, and NMDA receptors, and by regulating these substrates, it opposes the development of synaptic plasticity. Future work will determine whether STEP also plays a role in memory consolidation in brain regions outside of the amygdala. Additional studies should focus on STEP's contribution to CNS disorders, because of its critical substrates and high levels of expression in the striatum. As Confucius said "A journey of a thousand miles begins with a single step." The possibilities are only just beginning to be recognized and the path that clarifies the roles of STEP will be an exciting journey.

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CAPTIONS

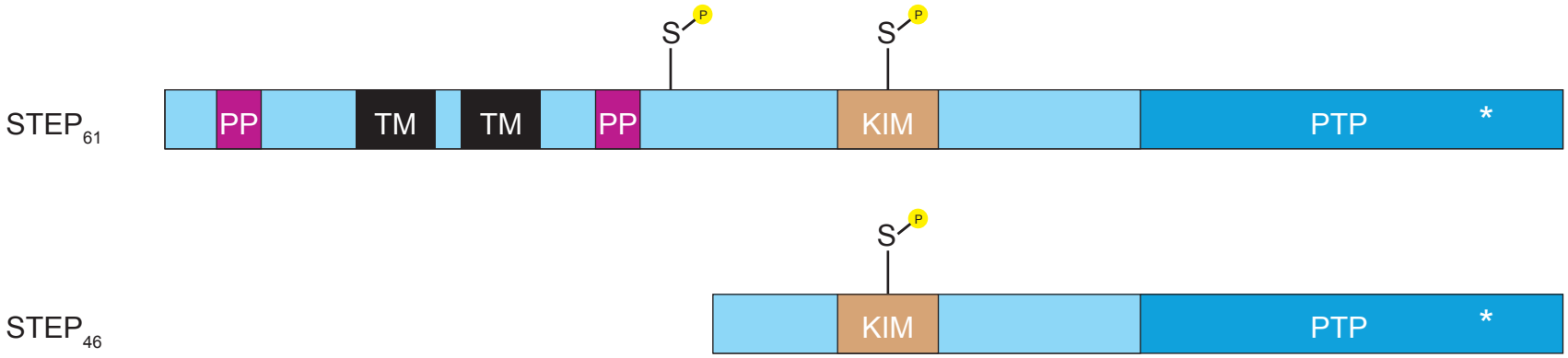
Figure 1: STEP structure. Alternative splicing produces two STEP isoforms: $STEP_{61}$ and $STEP_{46}$. $STEP_{46}$ is cytosolic while $STEP_{61}$ is targeted to the endoplasmic reticulum and the postsynaptic density. These two isoforms differ by an additional 172 amino acids at the N-terminus of $STEP_{61}$. This domain contains two transmembrane (TM) domains, two polyproline and two adjacent PEST domains (PP). One polyproline domain interacts with Fyn, while the PEST sequences are sites of potential cleavage. Domains that are shared by $STEP_{46}$ and $STEP_{61}$ include the binding site for ERK, the kinase interacting motif (KIM), and the approximately 280 amino acid phosphatase domain (PTP) containing an 11 amino acid catalytic site (*). $STEP_{61}$ has two serine PKA phosphorylation sites (S), whereas $STEP_{46}$ contains only the one within the KIM domain. Phosphorylation within the KIM domain sterically prevents the association of ERK with STEP, and leads to enzyme inactivation. The second serine site in $STEP_{61}$ is immediately adjacent to a PEST site, and is thought to activate proteolytic cleavage at that site.

Figure 2: STEP regulation. Dopamine stimulation of D1 receptors leads to cAMP synthesis, PKA activation and phosphorylation of STEP. Phosphorylation of the regulatory serine within the KIM domain prevents STEP from interacting with some substrates, such as ERK. Glutamate stimulation of NMDA receptors allows Ca^{2+} influx and activation of the serine phosphatase calcineurin leading to dephosphorylation of the regulatory KIM domain serine residue and thereby activation of STEP.

Figure 3: STEP dephosphorylates ERK, Fyn and the NMDA receptor complex. ERK, Fyn and NR2B subunit of the NMDA receptor are potential STEP substrates. Active ERK is required

for synaptic plasticity in all brain regions tested to date. In its activated state, ERK (1) phosphorylates cytoskeletal proteins, (2) regulates back-propagating action potentials, (3) stimulates protein synthesis, and (4) activates transcription. These processes work in parallel to promote synaptic plasticity. Fyn activation has also been implicated in synaptic plasticity through a variety of mechanisms including regulation of (5) glutamate receptor trafficking. Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor results in exocytosis of NMDA–containing endosomes.

Figure 4: STEP activation leads to abnormal NMDA receptor endocytosis in Alzheimer's disease. Aβ-peptide binding to the α7 nicotinic acetylcholine receptor (α7nAChR) leads to Ca^{2+} influx, calcineurin activation, and STEP dephosphorylation. Dephosphorylation activates STEP, which in turn inactivates Fyn. Fyn has been implicated in the phosphorylation of a regulatory tyrosine (Y^{1472}) on the NR2B subunit of the NMDA receptor that leads to exocytosis of this receptor. In the absence of Fyn-mediated tyrosine phosphorylation, the NMDA receptor is internalized by endocytosis. Active STEP opposes trafficking to the membrane by dephosphorylating Fyn and dephosphorylating the Y^{1472} site on the NR2B subunit.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PTP
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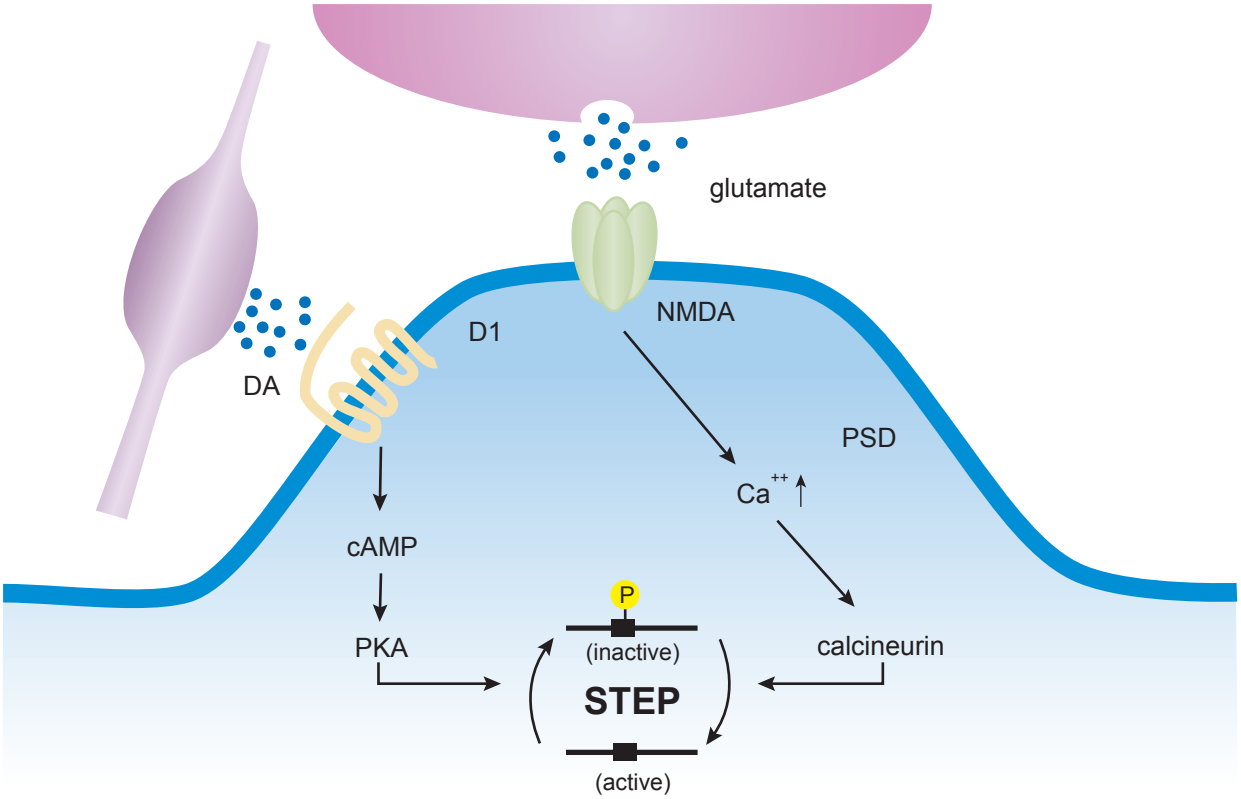
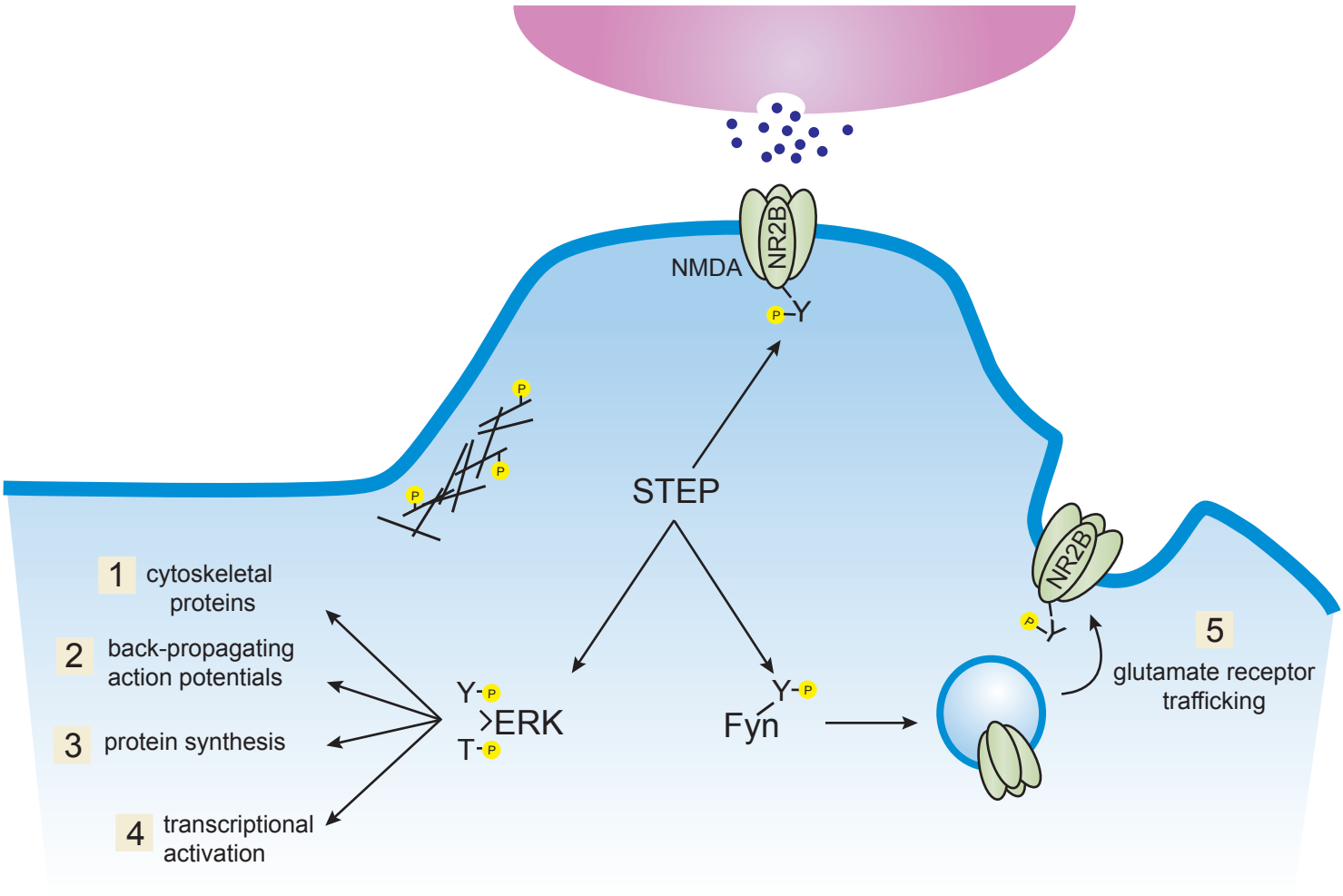
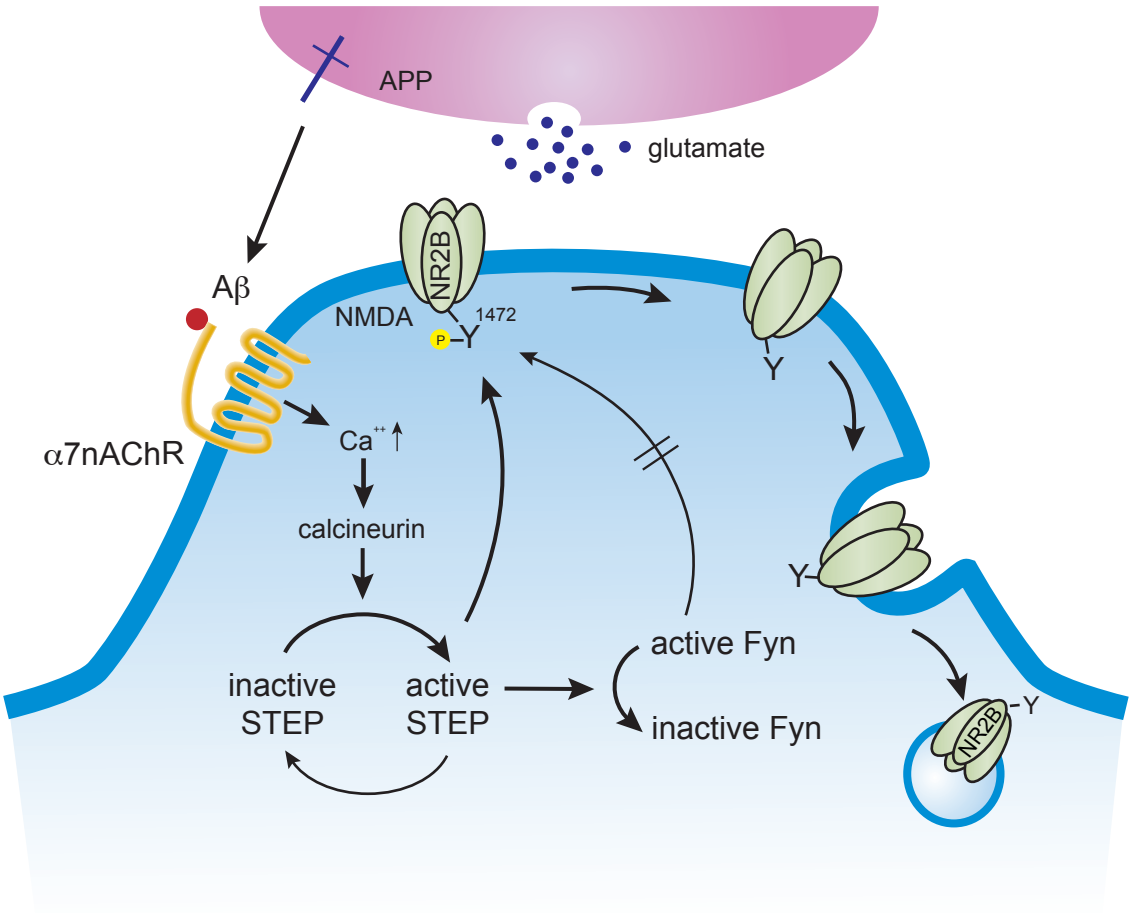

glutamate
NMDA
D1
DA
PSD
Ca++
cAMP
PKA
P
(inactive)
STEP
(active)
calcineurin


NMDA
NR2B
P–Y
STEP
1 cytoskeletal proteins
2 back-propagating action potentials
3 protein synthesis
4 transcriptional activation
Y-P
ERK
T-P
Y-P
Fyn
NR2B
5 glutamate receptor trafficking


APP
glutamate
Aβ
NMDA
NR2B
P
Y
1472
α7nAChR
Ca++
calcineurin
inactive
STEP
active
STEP
active Fyn
inactive Fyn
Y
Y
NR2B
Y