

# A STEP forward in neural function and degeneration

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**Key words:** tyrosine phosphatase, STEP, Alzheimer's disease, glutamate receptors, proteasome, ubiquitination

**Abbreviations:** AD, alzheimer's disease; AMPAR,  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor; MAPK, mitogen activated protein kinase; NMDAR, N-methyl-D-aspartate receptor; PKA, protein kinase A; STEP, striatal-enriched protein tyrosine phosphatase

Striatal-Enriched Phosphatase (STEP) is a brain-specific protein tyrosine phosphatase that plays a role in synaptic plasticity and has recently been implicated in neurodegenerative disease. STEP opposes the development of synaptic strengthening by dephosphorylating and inactivating key signaling proteins that include the MAP kinases ERK1/2 and p38, as well as the tyrosine kinase Fyn. STEP also dephosphorylates the GluR2 subunit of the AMPAR and the NR2B subunit of the NMDAR, which leads to internalization of the NR1/NR2B and GluR1/GluR2 receptors. STEP levels and activity are regulated through phosphorylation, local translation, ubiquitination and degradation and proteolytic cleavage. Here we review recent progress in understanding the normal regulation of STEP and how this regulation is disrupted in Alzheimer's disease, in which abnormally increased STEP levels and activity contribute to the cognitive deficits.

The ability to learn and react suitably to a changing environment depends on neuronal plasticity. Plasticity refers to the extent to which neurons can modify the structure and strength of their connections in response to synaptic activity (reviewed in ref. 1). While considerable research has focused on the mechanisms by which activity strengthens synaptic connections, less has concentrated on how synaptic connections are weakened. Recent work shows that Striatal-Enriched protein tyrosine Phosphatase (STEP) plays an important role in opposing synaptic strengthening.<sup>2</sup> STEP's molecular properties, regulation of its substrates ERK1/2, Fyn and the NMDAR complex, and its interactions with PKA and calcineurin have been reviewed in ref. 2 and are briefly mentioned here. Recent work has augmented our understanding of STEP: STEP activity also leads to the internalization of AMPARs,<sup>3</sup> and is regulated by proteolytic cleavage,<sup>4</sup> ubiquitin-proteasome degradation,<sup>4,5</sup> and local translation.<sup>3</sup> We discuss

these developments in light of their implications for plasticity and neurodegenerative disease.

## Expression and Functional Domains

STEP is expressed exclusively in the central nervous system where it is alternatively spliced into two major isoforms, STEP<sub>46</sub> and STEP<sub>61</sub>. STEP<sub>46</sub> is a cytosolic variant, whereas STEP<sub>61</sub> is targeted to the post-synaptic density and the endoplasmic reticulum by an additional 172 amino acids at its N-terminus.<sup>6</sup> This spatial localization is thought to be important to STEP function, especially as it relates to NMDAR and AMPAR trafficking.

STEP is enriched in medium spiny neurons of the striatum, where both STEP<sub>46</sub> and STEP<sub>61</sub> are expressed. STEP<sub>61</sub> is also found throughout the neocortex, hippocampus, amygdala and developing spinal cord.<sup>7</sup> Both STEP<sub>46</sub> and STEP<sub>61</sub> contain a catalytic protein tyrosine phosphatase (PTP) consensus sequence [I/V]HCxAGxxR[S/T]G and a kinase-interacting motif (KIM) necessary for interaction of STEP with its substrates. Unique to STEP<sub>61</sub> are two polyproline rich regions, as discussed below.

## STEP Substrates

**MAPKs: ERK1/2 and p38.** Several studies, reviewed elsewhere,<sup>2</sup> implicate STEP in the regulation of the MAPKs. Extracellular regulated kinase 1 and 2 (ERK1/2) activity induces and sustains synaptic strengthening through parallel processes. ERK1/2 promotes local protein translation<sup>8</sup> and gene transcription, and is involved in regulating spine stabilization and back-propagating action potentials (reviewed in ref. 9). ERK1/2 inhibition disrupts these processes. STEP binds ERK1/2 and p38 through its KIM domain and inactivates them by dephosphorylating the regulatory tyrosine in their activation loops.

ERK1/2 activity is significantly increased in the absence of STEP.<sup>10</sup> pERK1/2 is elevated in the striatum, area CA2 of the hippocampus, and the lateral and central amygdala in the brains of STEP knock-out (KO) mice. Moreover, STEP KO cultured neurons exhibit exaggerated pERK induction after pharmacological stimulation with DHPG, an agonist of the type I metabotropic glutamate receptor (mGluR) that leads to moderate ERK

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activation followed by synaptic depression. STEP's ability to bind to its substrates has been disrupted in an attempt to interfere with the consolidation of new memories. Infusion of a substrate-trapping, membrane permeable STEP<sub>46</sub> into the lateral amygdala blocked Pavlovian fear learning by preventing ERK1/2 translocation to the nucleus.<sup>11</sup>

The ability of STEP to regulate both ERK1/2 and p38 initially seemed paradoxical. Whereas ERK1/2 activation promotes cell survival and plasticity, p38 activation leads to cell death. A recent study clarified the mechanism by which STEP regulates both pro-survival and pro-cell-death pathways.<sup>4</sup> Extrasynaptic NMDAR stimulation, which leads to cell death, triggers local calpain-mediated cleavage of STEP<sub>61</sub> in its KIM domain. Cleavage prevents STEP from binding its substrates and causes selective activation of extrasynaptically concentrated p38.<sup>4</sup> In contrast, synaptic NMDAR stimulation leads to the ubiquitination and degradation of STEP<sub>61</sub> and predominantly ERK1/2 activation. Cell death is mitigated by preventing STEP cleavage, which suggests STEP plays a neuroprotective role.<sup>4</sup>

**Tyrosine kinase Fyn and NMDARs.** STEP regulates NMDAR trafficking through two parallel pathways. STEP acts as a brake on NMDAR exocytosis by dephosphorylating and inactivating Fyn (Y<sup>420</sup>). Fyn phosphorylates NR2B at tyr<sup>1472</sup>, leading to exocytosis of NMDARs to neuronal surfaces.<sup>12,13</sup> STEP<sub>61</sub> in particular seems important in this process as the presence of its polyproline region increases its affinity for Fyn 10-fold over STEP<sub>46</sub>. STEP also leads directly to clathrin-dependent endocytosis of the receptor complex by dephosphorylating NR2B (Y<sup>1472</sup>).<sup>14</sup>

**AMPA.** STEP also contributes to the regulation of GluR1/GluR2 AMPARs surface expression.<sup>3</sup> Pharmacological stimulation of group I mGluRs with DHPG normally promotes endocytosis of AMPARs and long-term depression (mGluR-LTD).<sup>15</sup> AMPAR endocytosis in NMDAR-LTD requires the dephosphorylation of a serine residue, whereas AMPAR endocytosis in mGluR-LTD requires the dephosphorylation of a tyrosine<sup>16</sup> and is completely blocked by inhibiting PTPs, suggesting that an unknown PTP is essential in mGluR-LTD.<sup>17</sup> We have recently shown that AMPAR surface expression is increased in STEP KO mice, whereas mGluR stimulation with DHPG no longer induces GluR1/2 endocytosis.<sup>18</sup> The introduction of a membrane-permeable WT STEP rescued the ability of DHPG to induce GluR1/2 internalization in the KO cultures.<sup>3</sup> These results implicate STEP as the PTP important in mGluR-dependent AMPAR trafficking. The identification of the specific Y residue on GluR2 that STEP dephosphorylates is subject of current investigation.

### STEP Regulation

**Phosphorylation.** PKA phosphorylation of S<sup>221</sup> in STEP's KIM domain prevents STEP interaction with its substrates. In striatal medium spiny neurons, D1 receptors couple positively to cAMP via G<sub>s</sub> to lead to the activation of PKA and consequent phosphorylation of STEP. Phosphorylation at S<sup>221</sup> can be reversed by Ca<sup>2+</sup> influx (via NMDAR or  $\alpha$ 7nAChR activation) and activation of the phosphatases, calcineurin and PP1. In this way, STEP acts as a coincidence detector for dopaminergic and glutamatergic or

cholinergic signaling. STEP's integration of these systems may be co-opted in psychostimulant addiction<sup>19</sup> and Alzheimer's disease.<sup>20</sup> Elevated A $\beta$  levels (1  $\mu$ M) activate  $\alpha$ 7nAChRs, which leads to calcineurin/PP1 activation, STEP dephosphorylation and activation, Fyn inactivation and NMDAR endocytosis. Disruption of the NMDAR system is thought to contribute to cognitive deficits. A substrate-trapping, catalytically inactive STEP blocked this A $\beta$ -induced NMDAR endocytosis in neuronal cultures and tissue slices.<sup>20</sup>

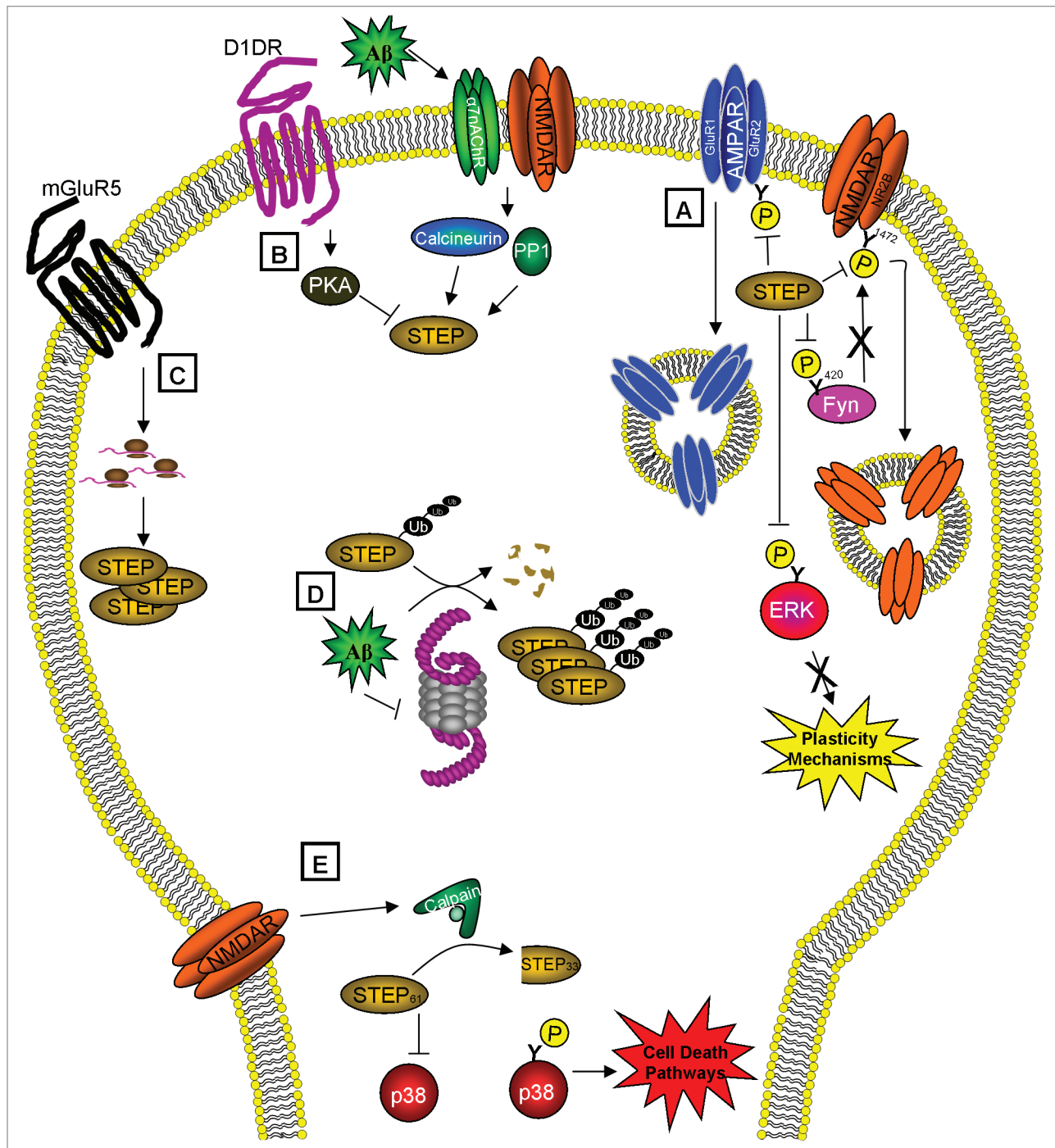
**Local translation.** The local translation of proteins in dendrites and spines is now known to be integral to the development of synaptic plasticity and its dysregulation is thought to be involved in the cognitive deficits present in Fragile X Syndrome.<sup>21</sup> STEP is rapidly translated in the amygdala within minutes after Pavlovian fear conditioning training, which suggested it might be translated locally.<sup>11</sup> We recently confirmed that STEP mRNA is present and locally translated upon stimulation of mGluR5 but not mGluR1 in synaptosomes, a preparation in which the presynaptic and postsynaptic compartments are isolated as a functional unit.<sup>3</sup> Moreover, translation was necessary for STEP-mediated endocytosis of AMPARs and required both ERK1/2 and PI3K pathways.

### A STEP Overboard: Dysregulation in Neurodegeneration

Alzheimer's disease (AD) is characterized by the presence of A $\beta$  plaques and hyperphosphorylated tau tangles. Emerging research suggests that soluble forms of A $\beta$  interfere with synapse function and may contribute to the pathophysiology of the disease.<sup>22</sup>

Our recent work further implicates STEP in the etiology of AD.<sup>5</sup> STEP level and activity are increased in human prefrontal cortex of patients with Alzheimer's disease as well as in cortex of an AD mouse model (Tg2576 mice). Moreover, 12-month but not 3-month old Tg2576 mice exhibit decreased pNR2B levels. Incubation with A $\beta$ -enriched medium (7PA2-CM) is sufficient to increase STEP levels in cultures and slices. Moreover, the A $\beta$ -enriched medium decreases the surface expression of NR1 and NR2B in WT cultured cortical neurons and cortical slices but has no effect in STEP KO cultures, which suggests that STEP is integral to the A $\beta$ -mediated NMDAR endocytosis. Interestingly, a follow-up experiment we performed after the publication of this paper shows that although the A $\beta$ -induced NMDAR internalization does not occur in STEP KO neuronal cultures, it is restored if wild-type STEP protein is added to the cultures prior to A $\beta$  stimulation.<sup>23</sup>

The mechanism by which A $\beta$  increases STEP<sub>61</sub> protein levels involves inhibition of the ubiquitin proteasome system.<sup>5</sup> Ubiquitinated STEP<sub>61</sub> accumulates in cortical cultures and slices treated with A $\beta$ -enriched medium and in the cortex of 12-month (but not 3-month) old Tg2576 mice. Pharmacological proteasome inhibition leads to the increase in STEP<sub>61</sub>, whereas neither translation nor transcription inhibitors block the increase. Overall, the study supports a model in which STEP<sub>61</sub> is normally degraded by the ubiquitin proteasome, but accumulates due to A $\beta$  blockade of the proteasome. STEP accumulation triggers the internalization



**Figure 1.** STEP functions and regulation. (A) STEP activity opposes the development of synaptic plasticity by dephosphorylating and inactivating ERK and Fyn and leading to the endocytosis of AMPA and NMDA receptors. STEP is itself regulated by (B) phosphorylation, (C) local translation, (D) ubiquitination and degradation and (E) proteolytic cleavage. (B) STEP is inactivated by PKA-phosphorylation after dopamine stimulation and activated by calcineurin/PP1 dephosphorylation of this residue after  $\alpha 7nAChR$  and NMDAR stimulation. (C) Stimulation of mGluR5 leads to local translation of STEP. (D) STEP is normally degraded by the ubiquitin proteasome system (UPS), but in the presence of A $\beta$ , the UPS is impaired, causing the accumulation of polyubiquitinated STEP. (E) Activation of extrasynaptic NMDARs triggers calpain to cleave STEP<sub>61</sub> into STEP<sub>33</sub>, which prevents STEP from regulating its substrates and leads to the release of p38 from inhibition and activation of cell death pathways. These regulatory pathways may contribute to the pathophysiology of Alzheimer's if disrupted, and also implicate STEP as an excellent target for drug discovery.

of glutamate receptors, the inactivation of Fyn and ERK1/2 and thereby contributes to the cognitive deficits that arise in this devastating illness. For a schematic summary of STEP function and regulation, see **Figure 1**.

### Concluding Remarks

STEP regulates the activity of MAPK, Fyn and the trafficking of NMDARs and AMPARs. Perhaps because of the importance of its substrates to normal neuronal function, regulation of STEP is itself proving enormously complex. Disruption at several levels—phosphorylation, translation, proteolysis, ubiquitination—may trigger a spiral into disease and pharmacological correction of

STEP activity may lead to a novel class of drugs for the treatment of Alzheimer's disease. Further research into these mechanisms promises to take us a step forward in our understanding of the biology of STEP and may suggest rational treatments of CNS disorders.

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