Cdk5 is a dynamo at the synapse

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Synaptic vesicle recycling is a highly regulated process that involves the coordinated function of several different presynaptic proteins including the dephosphins, whose dephosphorylation is important for triggering endocytosis. Subsequent rounds of endocytosis then depend on rephosphorylation of dephosphins. Recent work establishes Cdk5 as a dephosphin kinase whose function is necessary for synaptic vesicle endocytosis.

Communication between neurons occurs through highly specialized junctions called synapses. Synapses consist of the presynaptic cell, which releases neurotransmitter into the synaptic cleft, and the postsynaptic cell, which contains receptors that bind the neurotransmitter and initiate cellular responses. Neurotransmitters are stored in vesicles, which after excitation of the presynaptic terminal fuse with the presynaptic membrane and release neurotransmitter. Recycling of synaptic vesicles is then necessary for efficient synaptic transmission.

Synaptic vesicle endocytosis is a multistep process that requires several different presynaptic proteins at each stage[1–3]. Clathrin and AP180 are recruited to the presynaptic membrane where they function as building blocks for the coat that mediates budding of the recycled vesicle. Amphiphysin I and II function as scaffolds that help recruit dynamin I and synaptojanin to the clathrin coat. Dynamin I then mediates vesicle fission, most probably through its GTPase activity, whereas synaptojanin is required for synaptic vesicle uncoating after endocytosis. AP180, amphiphysin I and II, dynamin I and synaptojanin have all been classified as dephosphins, because it is their phosphorylation status that regulates synaptic vesicle recycling.

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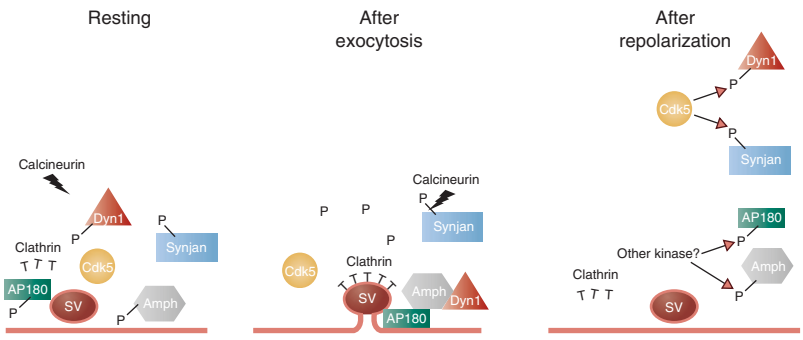

Figure 1 The phosphorylation/de-phosphorylation cycle in synaptic vesicle endocytosis. In the resting state, synaptic vesicles are stored at the presynaptic terminal and are primed for exocytosis. Dephosphin proteins are phosphorylated (left). Upon depolarization and immediately after exocytosis, the calcium-dependent phosphatase calcineurin rapidly dephosphorylates the dephosphin proteins and triggers endocytosis of the synaptic vesicle (middle). After repolarization, the neuron returns to a resting state as Cdk5 rephosphorylates the dephosphins dynamin I and synaptojanin. An unknown kinase is responsible for the rephosphorylation of amphiphysins and AP180 (right).

Although dephosphins are structurally and functionally diverse and have been implicated in different steps of synaptic vesicle endocytosis, they all share one common feature: each dephosphin undergoes a cycle of phosphorylation and dephosphorylation during a single round of synaptic vesicle endocytosis. In the resting state, dephosphins are phosphorylated. After excitation of the presynaptic terminal, however, the calcium-dependent phosphatase calcineurin coordinately dephosphorylates the dephosphins, triggering endocytosis. For subsequent rounds of endocytosis, it is essential that dephosphins are rephosphorylated. To date, the kinases responsible for this event have remained obscure. Now, on page 701 of this issue, Tan *et al.* identify the small serine/threonine kinase Cdk5 as a dephosphin kinase[4].

Although previous work has established that Cdk5 is essential during neuronal migration and is involved in neurodegenerative diseases[5], several lines of evidence also suggest that Cdk5 is an attractive candidate for a dephosphin kinase: first, the *Dictyostelium discoideum* Cdk5 homologue is invloved in endocytosis[6]; second, the *Saccharomyces cerevisiae* Cdk5 homologue Pho85 phosphorylates the amphiphysin homologue Rvs167 (ref. 7); third, Cdk5 is enriched at the synapse in mammals[8], where it has been shown to phosphorylate amphiphysin I (ref. 9); finally, Cdk5 also has several other presynaptic substrates, such as Munc-18 (ref. 10), synapsin 1 (ref. 11) and the α1A subunit of P/Q-type calcium channels[8].

Now, Tan *et al.*[4] add the dephosphin dynamin I to this list and show it is phosphorylated by Cdk5 at Ser 774 and Ser 778. Protein kinase C, which has been suggested as another candidate dephosphin kinase[12], phosphorylates dynamin I at Ser 795. In their study, Tan *et al.*

use mass spectrometry analysis of endogenous dynamin I to show that it is phosphorylated at Ser 774 and Ser 778 *in vivo*, but not at Ser 795. Furthermore, analysis with phosphospecific antibodies demonstrated that phosphorylation of dynamin I at Ser 774 and Ser 778 is increased after repolarization. Together, these results suggest that Ser 774 and Ser 778 are the physiologically relevant sites for a dephosphin kinase.

More direct evidence for the role of Cdk5 during synaptic vesicle endocytosis comes from the finding that the Cdk5 inhibitor roscovitine blocks rephosphorylation of dynamin I after repolarization of synaptosomes. Most interestingly, the authors found that although the initial round was not affected, roscovitine significantly inhibited the second round of synaptic vesicle endocytosis. In addition, overexpression of a dominant-negative Cdk5 mutant in cultured cerebellar granule neurons also inhibited synaptic vesicle recycling, strongly suggesting that Cdk5 is a dephosphin kinase essential for promoting multiple rounds of endocytosis.

Tan *et al.* also showed that roscovitine inhibits rephosphorylation of another dephosphin, synaptojanin, suggesting that the role of Cdk5 as a dephosphin kinase extends beyond its effects on dynamin I to include other dephosphins as substrates. However, phosphorylation of both AP180 and amphiphysin I was unaffected by roscovitine treatment. This is a surprising finding, given that amphiphysin I is a substrate for Cdk5 in mammals and that phosphorylation of Rvs167 by Pho85 is important for endocytosis in yeast. Thus, the model provided by Tan *et al.* suggests a fundamental role for Cdk5 in rephosphorylation of some dephosphins after repolarization and also leaves the door open for an (as yet) unidentified kinase (Fig. 1).

Although this study furthers our knowledge of synaptic vesicle endocytosis by establishing a role for Cdk5, the precise function of the phosphorylation/dephosphorylation cycle for each dephosphin and how the phosphorylation status of these few proteins ultimately triggers endocytosis remains unknown. It has been suggested that phosphorylation of dephosphins might regulate protein–protein interactions and the formation of complexes containing the dephosphins[13]. In contrast to an earlier study showing that dynamin I phosphorylation inhibits binding to amphiphysin I *in vitro*[13], Tan *et al.* find that dynamin I phosphorylation does not affect binding to amphiphysin I, but instead reduces the ability of dynamin I to associate with membrane phospholipids. This suggests that the phosphorylation status of a dephosphin can regulate its recruitment to the synaptic membrane by directly affecting its association with membrane lipids. Tan *et al.* add yet another piece to this already complicated puzzle with data suggesting that phosphorylation by Cdk5 also increases the GTPase activity of dynamin I.

Future studies will need to investigate the exact function of phosphorylation and dephosphorylation of each dephosphin. A temporally inducible Cdk5 loss-of-function system will become a necessary tool in these studies for several reasons: first, this system can be used to confirm the role of Cdk5 as a dephosphin kinase and should help to clear up exactly which dephosphins are the relevant Cdk5 substrates; second, dephosphin protein complexes and their functions during different stages of endocytosis could be readily studied in the absence of phosphorylation by one of the dephosphin kinases. Non-phosphorylatable mutants of the different dephosphins should also help to define the relevant phosphorylation sites and their functions.

Despite several unanswered questions, the study by Tan *et al.* establishes Cdk5 as a major player at the synapse and suggests that multiple Cdk5 substrates are involved in synaptic vesicle recycling. One intriguing possibility that remains to be thoroughly examined is whether Cdk5 or its essential activators, p35 and p39, are directly regulated during the synaptic vesicle cycle. This could be accomplished by a post-translational modification that directly modulates Cdk5 activity, or alters localization of the kinase or its activators. Another possibility could also be that synaptic activity regulates changes in the levels of Cdk5 or activator protein. A transient alteration in Cdk5 activity induced by depolarization at the presynaptic terminal would provide an attractive model to help explain why dephosphins are specifically rephosphorylated only after repolarization. Interestingly, it has been suggested recently that depolarization can cause a decrease in Cdk5 activity, although the precise mechanism is not known[14]. It has also been shown, however, that activation of group I metabotropic glutamate receptors transiently increases Cdk5 activity[15]. Either way, direct regulation of Cdk5 would be exciting in light of its multiple substrates at the synapse. □

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New dimensions in cell migration

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Cell migration research is taking some intriguing new turns. Emerging studies are revealing marked differences in the way that cells migrate in two versus three dimensions, plasticity in their migration mechanisms and diversity in the contribution of different Rho family GTPases that drive various modes of migration.

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Studies of cell migration are moving into the next dimension and causing us to re-assess the mechanisms that mediate motility. In 3D environments, such as collagen gels, acellular matrices, or slice cultures, the way that cells move and the morphologies they adopt differ markedly from those seen with dissociated cells migrating on the more commonly used planar substrata[1–4]. The plasticity of cell migration has become evident through the adaptation strategies that permit migration to occur in response to new environments or stresses[2,3].