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Regulation of the phosphatase PP2B by protein–protein interactions

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Protein dephosphorylation is important for regulating cellular signaling in a variety of contexts. Protein phosphatase-2B (PP2B), or calcineurin, is a widely expressed serine/threonine phosphatase that acts on a large cross section of potential protein substrates when activated by increased levels of intracellular calcium in concert with calmodulin. PxIxIT and LxVP targeting motifs are important for maintaining specificity in response to elevated calcium. In the present study, we describe the mechanism of PP2B activation, discuss its targeting by conserved binding motifs and review recent advances in the understanding of an A-kinase anchoring protein 79/PP2B/protein kinase A complex's role in synaptic long-term depression. Finally, we discuss potential for targeting PP2B anchoring motifs for therapeutic benefit.

PP2B targeting by substrate interaction motifs and anchoring proteins

The present study focuses on how protein–protein interactions between protein phosphatase-2B (PP2B), also known as calcineurin, and substrates and anchoring proteins influence physiological processes controlled by this enzyme.

Reversible protein phosphorylation is perhaps the most well-studied post-translational modification [1]. It has been shown to be vital for almost all cellular signaling pathways, regulating signaling events in response to input from extracellular cues or from modulatory signaling cross-talk. Attachment of phosphate groups to protein substrates by kinases is reversed by the action of protein phosphatases, which are often specific for phosphotyrosine or phosphoserine/threonine residues.

PP2B is a ubiquitous serine (Ser)/threonine (Thr) phosphatase that is activated by elevated calcium levels and subsequent activation of calmodulin (CaM) [2]. The PP2B holoenzyme is a heterodimer that consists of a catalytic A subunit and a regulatory B subunit. When activated, this holoenzyme includes CaM in its calcium-bound state. Elevation of calcium and activation of CaM cause a structural rearrangement that allows CaM to bind to an α-helix in the A subunit of PP2B and remove an autoinhibitory helix from the active site of the phosphatase (Figure 1A) [3].

Because PP2B has the capacity to promiscuously dephosphorylate most solvent-exposed Ser/Thr residues, the controlled activation and inactivation by calcium transients is important for signaling specificity [4]. Moreover, substrate specificity is controlled by a variety of protein–protein interactions with anchoring proteins and allosteric recognition sequences on PP2B substrates [5–7]. In particular, the A-kinase anchoring protein 79 (AKAP79, AKAP150 in rodents; products of the *AKAP5* gene), interacts with both PP2B and protein kinase A (PKA) [8,9]. This co-localization of a kinase and a phosphatase permits exquisite control of post-translationally mediated signaling events [10,11].

PP2B's interactions with binding partners ensure that this broad spectrum phosphatase can have specific roles in diverse physiological contexts, such as neurotransmission, cardiac signaling, immune responses, and insulin signaling. We will describe the molecular basis for interactions between PP2B and anchoring proteins/substrates, and review how these interactions control cellular signaling events.

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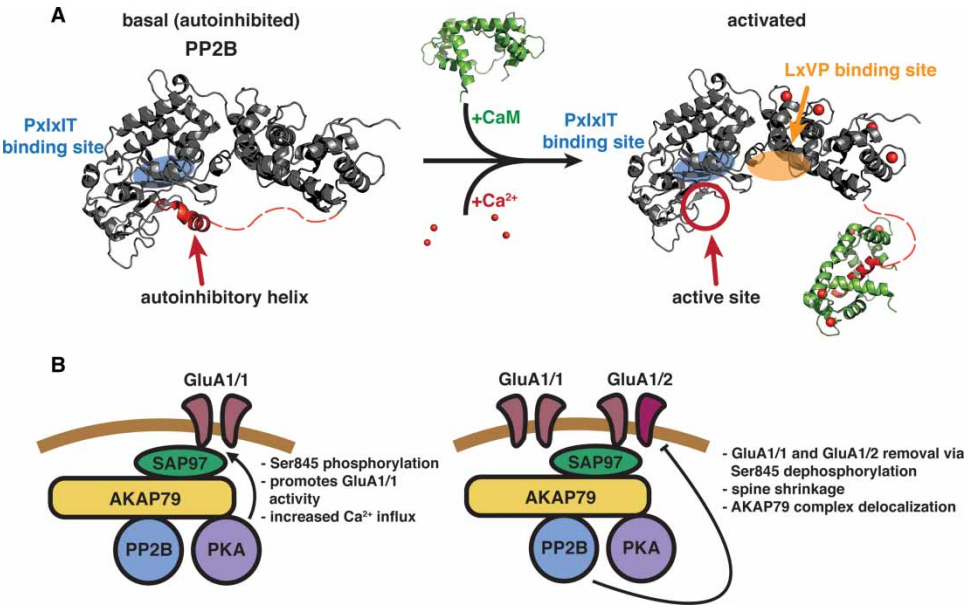

Figure 1. (**A**) Schematic of PP2B (gray) activation by CaM (green) and calcium. Structural rearrangement of the autoinhibitory helix (red) allows catalytic activity. PxIxIT (blue) and LxVP (orange) binding surfaces are indicated, as well as the approximate location of the active site (red circle). (**B**) AKAP79/SAP97-mediated complex of GluA1-containing AMPA receptors, PP2B and PKA. Upon elevation of calcium, PKA and PP2B work in a distinct manner to promote long-term depression through regulation of AMPA receptors.

Anchoring and substrate interactions

The catalytic subunit of PP2B resembles the catalytic subunit of protein phosphatase-1 (PP1) [5,12]. However, while PP1 holoenzymes are formed via interactions with a large variety of regulatory and targeting subunits [13], PP2B holoenzymes can be formed with different combinations of three catalytic A-subunit isoforms (α, β, and γ) and two regulatory B-subunit isoforms (type 1 and type 2) [3,14,15]. These isoforms lack both the structural and functional diversity of targeted PP1 holoenzymes; therefore, the varied roles of PP2B in biological processes are often mediated by a similarly diverse range of interacting proteins.

However, the majority of these interactions are through two distinct surfaces on PP2B. The best-characterized surface is composed of a β-sheet on the catalytic subunit. This β-sheet interacts with a conserved motif known as the PxIxIT motif. The PxIxIT motif forms a short β-strand that interacts with β-strand 14 of the catalytic subunit and extends the β-sheet (Figure 1A) [16,17].

Several interacting proteins contain the PxIxIT motif, notably the canonical PP2B substrate nuclear factor of activated T-cells (NFAT) [18] and AKAP79/150 (Table 1) [19,20]. Other proteins that contain PxIxIT motifs include the scaffolding protein regulator of calcineurin 1 (RCAN1) [21] and the TWIK-related spinal cord potassium channel (TRESK) [5]. The primary function of the PxIxIT motif is thought to be to spatially recruit PP2B to nearby substrates. Structures of PP2B in complex with a synthetic PxIxIT peptide [17], as well as a PxIxIT peptide analog derived from AKAP79 [16], have been solved by crystallography.

A more recently characterized PP2B-interacting motif is termed the LxVP motif. This motif binds near the interface of the catalytic and regulatory subunits of PP2B and forms important contacts with both subunits (Figure 1A) [6,22]. This interface is also spatially removed from the active site of the phosphatase, although it has been suggested that it serves to optimally place phosphosubstrates for efficient dephosphorylation [22]. This is supported by the observation that LxVP motifs only bind to activated PP2B (in the presence of calcium and CaM). In addition, the LxVP-binding site overlaps with the binding site for the immunosuppressant/immunophilin complexes cyclosporin/cyclophilin and FK506/FKBP12 [22]. A recent study suggests that the main mechanism of action for these immunosuppressants is to compete for binding with the LxVP motif present in NFAT. This conserved LxVP motif seems to have a looser consensus sequence than the PxIxIT, and the key requirements for binding have not yet been fully defined [5,22].

Table 1 PP2B-binding motifs in anchoring proteins and substrates

PP2B-binding partner	Demonstrated phosphosubstrate	PxIxIT motif identified	LxVP motif identified	References
Cain/Cabin1	Yes	Yes	No	[61]
NFAT	Yes	Yes	Yes	[62]
RCAN1	Yes	Yes	Yes	[21]
AKAP79	Yes	Yes	No	[19,20]
KSR2	Yes	No	Yes	[24]
TRESK	Yes	Yes	Yes	[63,64]
PKA-RIIα	Yes	No	Yes	[23]
DRP1	Yes	No	Yes	[65]
A238L	No	Yes	Yes	[22]
Myosin phosphatase	Yes	Yes	No	[66]
Dynamin 1	Yes	Yes	No	[67]

The LxVP motif was originally identified in a region of the RIIα regulatory subunit of PKA that was used as a substrate peptide for the phosphatase [23]. However, the LxVP sequence was not recognized as a consensus motif at that time. Subsequent analyses revealed that NFAT contains an LxVP motif, as do other PP2B substrates. In addition, a peptide derived from a viral protein that contains both an LxVP motif and a PxIxIT motif was co-crystallized with PP2B, and the resulting structure has shed light on the binding site of the LxVP motif (Table 1) [22].

Proteins that contain LxVP motifs have been shown to use them for various purposes. NFAT family members use the LxVP motif to stimulate dephosphorylation of phosphosites located between the LxVP motif and the PxIxIT motif [5]. The scaffold protein KSR2 appears to only have an LxVP motif and lacks a PxIxIT, and this promotes dephosphorylation of upstream distal Ser and Thr residues [24]. Because of the flexibility inherent in the LxVP motif, it is likely that most proteins contain regions of disorder surrounding the LxVP motif that act analogously to the disorder in RII, allowing a range of nearby phosphosites to occupy a constrained yet flexible conformational space so that multiple sites can be dephosphorylated by a single-anchored PP2B molecule [22].

As more LxVP motifs are identified in other proteins, undoubtedly the function and consensus of this sequence will be more well understood. The combination of multiple binding motifs for a single phosphatase allows fine-tuning of binding affinities under different enzymatic states, while also possibly influencing substrate selectivity. The potential for the LxVP motif to fine-tune the activity and specificity of PP2B may be important for understanding how anchored PP2B acts in regulating synaptic transmission.

Postsynaptic signaling

Synaptic plasticity — the ability of synapses to undergo changes in the strength of their response to neurotransmitter release — is believed to underlie aspects of contextual learning and memory [25,26]. Many of these changes are mediated by *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors on the postsynaptic neuron, which are modulated by phosphorylation events. These covalent modifications promote changes in the number of receptors at the synapse, as well as changes to receptor ion permeability [27]. AMPA receptors are tetrameric channels that are composed of some combination of four different subunit isoforms — GluA1–4. Homomeric GluA1/1 receptors are calcium permeable, while heteromeric receptors that contain GluA2 are considered to be calcium impermeable [28].

PP2B has been suggested to be particularly important for modulating the phosphorylation state of Ser845 of the GluA1 subunit of the AMPA receptor in the hippocampal region of the brain [29–31]. Phosphorylation of Ser845 increases ion permeability and also results in increased trafficking to the postsynaptic density [29]. PP2B has long been implicated in dephosphorylation of this site in response to high-frequency stimulation and is targeted to AMPA receptors by a protein complex that involves AKAP79 and, the membrane-associated

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guanylate kinase protein, synapse-associated protein 97 (SAP97) [29,32]. SAP97 is a scaffolding protein that interacts with the GluA1 subunit of the AMPA receptor and with AKAP79 to form a macromolecular complex that serves as an important signaling node in the postsynaptic density [33,34].

While the dephosphorylation of Ser845 has been known to be important for a form of synaptic plasticity called NMDA receptor-dependent long-term depression (LTD), the exact molecular mechanisms have not been clear [35,36]. Early work implicated a PP2B/PP1 cascade in regulating this type of LTD; however, previous studies suggest that PP2B directly dephosphorylates Ser845 [37–40]. Recent work highlights the importance of the AKAP79/150 complex containing both PKA and PP2B in inducing LTD in the CA1 region of the hippocampus [41–43]. It is also important to note that a role of AKAP79/150 interactions with GABA receptors at inhibitory synapses has also been proposed. Therefore, AKAP79/150 may be a more general synaptic anchoring protein that co-ordinates the differential localization of PP2B and a variety of other kinases to various synaptic sites [44].

After initial studies with AKAP150 knockout mice [36], several knock-in mouse models were generated which express forms of the anchoring protein that lack binding sites for PKA (ΔPKA) [35], protein kinase C (ΔPKC) [45], or PP2B (ΔPIX) [30]. These mouse models have been used to study a variety of physiological arenas, such as vascular smooth muscle [45,46], cardiac function [47,48], and glucose homeostasis [49]. Recently, the ΔPIX and ΔPKA mouse lines have been used to show that in the postsynaptic density, AKAP79/150-anchored PKA is necessary to phosphorylate homomeric GluA1 AMPA receptors at Ser845 and recruit them in rapid response to calcium influx through NMDA receptors. These homomeric calcium permeable GluA1 receptors are recruited to the synapse and augment the later calcium response to glutamate in order to efficiently activate AKAP79/150-anchored PP2B [42]. This PP2B activation promotes GluA1 dephosphorylation to remove both the homomeric GluA1 AMPA receptors and the heteromeric ionotropic glutamate receptors 1/2, resulting in an overall decrease in synaptic strength [42]. This activated PP2B has also been shown to be important in spine shrinkage and trafficking of AKAP79/150 and MAGUK protein complexes away from the postsynaptic density [19,33,50,51].

Another long-standing puzzle in neuronal signaling has been the relative activity of the Ca^{2+}/CaM-dependent protein kinase II (CaMKII) and PP2B in response to postsynaptic influxes of calcium. Both enzymes are activated by calcium/CaM and have many overlapping substrates, such as the GluA1 subunit of the AMPA receptor, the NMDA receptor, and other postsynaptic targets [50,52]. How these enzymes are differentially regulated may be explained not only by their differential sensitivity to calcium, but also by their localization within the postsynaptic density by anchoring and substrate interactions. In addition, previous studies have implicated neuronal pools of AKAP79-anchored PP2B in dephosphorylation of NFAT in response to calcium entry via the L-type calcium channel [16,19].

As more is learned about the functional effects of PP2B anchoring, light will be shed on these questions as well as on the role of PP2B in the other diverse physiological events it helps control.

Conclusion: targeting PP2B for therapeutics

PP2B is an important therapeutic target for cyclosporin and FK506, immunosuppressants used clinically for organ transplant patients as well as to treat psoriasis and rheumatoid arthritis [53–55]. However, its role in synaptic plasticity, as well as in other processes such as insulin signaling [49], and cardiac function [56–58] make PP2B an attractive potential target for other conditions. In order to effectively target specific pathways, PP2B's interactions with anchoring proteins, such as AKAP79 or specific phosphosubstrates, need to be disrupted. This requires a detailed understanding of what the structural basis for these interactions is, as well as the relative functions of the PxIxIT and LxVP-binding motifs.

Several small molecules that disrupt PP2B interactions have already been developed, including the INCA compounds [59] and the VIVIT peptide [17,60], both of which disrupt interactions with the PxIxIT motif. The fact that binding sites for the LxVP motif and the immunophilin complexes overlap suggests that the targeted disruption of PP2B's interaction with the LxVP motif is a promising avenue for specific therapeutic treatment [22]. Because PP2B has a wide range of potential targets, targeting the catalytic site of PP2B is likely to have a plethora of undesirable effects, whereas targeting interaction motifs will provide the best specificity. Only a more thorough understanding of the function and molecular details of these interaction motifs will allow such an approach to be successful.

Abbreviations

AKAP, A-kinase anchoring protein; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaM, calmodulin; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; LTD, long-term depression; NFAT, nuclear factor of activated T-cells; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; PP1, protein phosphatase-1; PP2B, protein phosphatase-2B; RCAN1, regulator of calcineurin 1; SAP97, synapse-associated protein 97; Ser, serine; Thr, threonine; TRESK, TWIK-related spinal cord potassium channel.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Pawson, T. and Scott, J.D. (2005) Protein phosphorylation in signaling – 50 years and counting. *Trends Biochem. Sci.* **30**, 286–290 doi:10.1016/j.tibs.2005.04.013
2 Stewart, A.A., Ingebritsen, T.S., Manalan, A., Klee, C.B. and Cohen, P. (1982) Discovery of a Ca^{2+}- and calmodulin-dependent protein phosphatase. *FEBS Lett.* **137**, 80–84 doi:10.1016/0014-5793(82)80319-0
3 Li, H., Rao, A. and Hogan, P.G. (2011) Interaction of calcineurin with substrates and targeting proteins. *Trends Cell. Biol.* **21**, 91–103 doi:10.1016/j.tcb.2010.09.011
4 Mehta, S., Aye-Han, N.-N., Ganesan, A., Oldach, L., Gorshkov, K. and Zhang, J. (2014) Calmodulin-controlled spatial decoding of oscillatory Ca^{2+} signals by calcineurin. *eLife* **3**, e03765 doi:10.7554/eLife.03765
5 Roy, J. and Cyert, M.S. (2009) Cracking the phosphatase code: docking interactions determine substrate specificity. *Sci. Signal.* **2**, re9 doi:10.1126/scisignal.2100re9
6 Rodríguez, A., Roy, J., Martínez-Martínez, S., López-Maderuelo, M.D., Niño-Moreno, P., Ortí, L. et al. (2009) A conserved docking surface on calcineurin mediates interaction with substrates and immunosuppressants. *Mol. Cell* **33**, 616–626 doi:10.1016/j.molcel.2009.01.030
7 Roy, J., Li, H., Hogan, P.G. and Cyert, M.S. (2007) A conserved docking site modulates substrate affinity for calcineurin, signaling output, and in vivo function. *Mol. Cell* **25**, 889–901 doi:10.1016/j.molcel.2007.02.014
8 Coghlan, V.M., Perrino, B.A., Howard, M., Langeberg, L.K., Hicks, J.B., Gallatin, W.M. et al. (1995) Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* **267**, 108–111 doi:10.1126/science.7528941
9 Carr, D.W., Hausken, Z.E., Fraser, I.D., Stofko-Hahn, R.E. and Scott, J.D. (1992) Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. Cloning and characterization of the RII-binding domain. *J. Biol. Chem.* **267**, 13376–13382 PMID: 1618839
10 Tasken, K. and Aandahl, E.M. (2004) Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol. Rev.* **84**, 137–167 doi:10.1152/physrev.00021.2003
11 Langeberg, L.K. and Scott, J.D. (2015) Signalling scaffolds and local organization of cellular behaviour. *Nat. Rev. Mol. Cell Biol.* **16**, 232–244 doi:10.1038/nrm3966
12 Choy, M.S., Hieke, M., Kumar, G.S., Lewis, G.R., Gonzalez-DeWhitt, K.R., Kessler, R.P. et al. (2014) Understanding the antagonism of retinoblastoma protein dephosphorylation by PNUTS provides insights into the PP1 regulatory code. *Proc. Natl Acad. Sci. USA* **111**, 4097–4102 doi:10.1073/pnas.1317395111
13 Bollen, M. (2001) Combinatorial control of protein phosphatase-1. *Trends Biochem. Sci.* **26**, 426–431. doi:10.1016/S0968-0004(01)01836-9
14 Guerini, D. and Klee, C.B. (1991) Structural diversity of calcineurin, a Ca^{2+} and calmodulin-stimulated protein phosphatase. *Adv. Protein Phosphatases* **6**, 391–410
15 Kuno, T., Takida, T., Hirai, M., Ito, A., Mukai, H. and Tanaka, C. (1989) Evidence for a second isoform of the catalytic subunit of calmodulin-dependent prtoein phosphatase (calcineurin A). *Biochem. Biophys. Res. Commun.* **165**, 1352–1358 doi:10.1016/0006-291X(89)92752-6
16 Li, H., Pink, M.D., Murphy, J.G., Stein, A., Dell'acqua, M.L. and Hogan, P.G. (2012) Balanced interactions of calcineurin with AKAP79 regulate Ca^{2+}–calcineurin–NFAT signaling. *Nat. Struct. Mol. Biol.* **19**, 337–345 doi:10.1038/nsmb.2238
17 Li, H., Zhang, L., Rao, A., Harrison, S.C. and Hogan, P.G. (2007) Structure of calcineurin in complex with PVIVIT peptide: portrait of a low-affinity signalling interaction. *J. Mol. Biol.* **369**, 1296–1306 doi:10.1016/j.jmb.2007.04.032
18 Park, S., Uesugi, M. and Verdine, G.L. (2000) A second calcineurin binding site on the NFAT regulatory domain. *Proc. Natl Acad. Sci. USA* **97**, 7130–7135. doi:10.1073/pnas.97.13.7130
19 Oliveria, S.F., Dell'Acqua, M.L. and Sather, W.A. (2007) AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca^{2+} channel activity and nuclear signaling. *Neuron* **55**, 261–275 doi:10.1016/j.neuron.2007.06.032
20 Dell'Acqua, M.L., Dodge, K.L., Tavalin, S.J. and Scott, J.D. (2002) Mapping the protein phosphatase-2B anchoring site on AKAP79. Binding and inhibition of phosphatase activity are mediated by residues 315–360. *J. Biol. Chem.* **277**, 48796–48802 doi:10.1074/jbc.M207833200
21 Mehta, S., Li, H., Hogan, P.G. and Cunningham, K.W. (2009) Domain architecture of the regulators of calcineurin (RCANs) and identification of a divergent RCAN in yeast. *Mol. Cell. Biol.* **29**, 2777–2793 doi:10.1128/MCB.01197-08

Biochemical Society Transactions (2016) **44** 1313–1319
DOI: 10.1042/BST20160150

22 Grigoriu, S., Bond, R., Cossio, P., Chen, J.A., Ly, N., Hummer, G. et al. (2013) The molecular mechanism of substrate engagement and immunosuppressant inhibition of calcineurin. *PLoS Biol.* **11**, e1001492 doi:10.1371/journal.pbio.1001492

23 Blumenthal, D.K., Takio, K., Hansen, R.S. and Krebs, E.G. (1986) Dephosphorylation of cAMP-dependent protein kinase regulatory subunit (type II) by calmodulin-dependent protein phosphatase. *J. Biol. Chem.* **261**, 8140–8145 PMID: 3013843

24 Dougherty, M.K., Ritt, D.A., Zhou, M., Specht, S.I., Monson, D.M., Veenstra, T.D. et al. (2009) KSR2 is a calcineurin substrate that promotes ERK cascade activation in response to calcium signals. *Mol. Cell* **34**, 652–662 doi:10.1016/j.molcel.2009.06.001

25 Sheng, M. and Kim, M.J. (2002) Postsynaptic signaling and plasticity mechanisms. *Science* **298**, 776–780 doi:10.1126/science.1075333

26 Huganir, R.L. and Nicoll, R.A. (2013) AMPARs and synaptic plasticity: the last 25 years. *Neuron* **80**, 704–717 doi:10.1016/j.neuron.2013.10.025

27 Malinow, R. and Malenka, R.C. (2002) AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 doi:10.1146/annurev.neuro.25.112701.142758

28 Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R. et al. (2009) Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. *Neuron* **62**, 254–268 doi:10.1016/j.neuron.2009.02.027

29 Tavalin, S.J., Colledge, M., Hell, J.W., Langeberg, L.K., Huganir, R.L. and Scott, J.D. (2002) Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *J. Neurosci.* **22**, 3044–3051 PMID: 11943807

30 Sanderson, J.L., Gorski, J.A., Gibson, E.S., Lam, P., Freund, R.K., Chick, W.S. et al. (2012) AKAP150-anchored calcineurin regulates synaptic plasticity by limiting synaptic incorporation of Ca^{2+}-permeable AMPA receptors. *J. Neurosci.* **32**, 15036–15052 doi:10.1523/JNEUROSCI.3326-12.2012

31 Joiner, M.-I.A., Lisé, M.-F., Yuen, E.Y., Kam, A.Y.F., Zhang, M., Hall, D.D. et al. (2010) Assembly of a β_2-adrenergic receptor—GluR1 signalling complex for localized cAMP signalling. *EMBO J.* **29**, 482–495 doi:10.1038/emboj.2009.344

32 Jurado, S., Biou, V. and Malenka, R.C. (2010) A calcineurin/AKAP complex is required for NMDA receptor–dependent long-term depression. *Nat. Neurosci.* **13**, 1053–1055 doi:10.1038/nn.2613

33 Robertson, H.R., Gibson, E.S., Benke, T.A. and Dell'Acqua, M.L. (2009) Regulation of postsynaptic structure and function by an A-kinase anchoring protein-membrane-associated guanylate kinase scaffolding complex. *J. Neurosci.* **29**, 7929–7943 doi:10.1523/JNEUROSCI.6093-08.2009

34 Colledge, M., Dean, R.A., Scott, G.K., Langeberg, L.K., Huganir, R.L. and Scott, J.D. (2000) Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* **27**, 107–119 doi:10.1016/S0896-6273(00)00013-1

35 Weisenhaus, M., Allen, M.L., Yang, L., Lu, Y., Nichols, C.B., Su, T. et al. (2010) Mutations in AKAP5 disrupt dendritic signaling complexes and lead to electrophysiological and behavioral phenotypes in mice. *PLoS ONE* **5**, e10325 doi:10.1371/journal.pone.0010325

36 Tunquist, B.J., Hoshi, N., Guire, E.S., Zhang, F., Mullendorff, K., Langeberg, L.K. et al. (2008) Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proc. Natl Acad. Sci. USA* **105**, 12557–12562 doi:10.1073/pnas.0805922105

37 Morishita, W., Connor, J.H., Xia, H., Quinlan, E.M., Shenolikar, S. and Malenka, R.C. (2001) Regulation of synaptic strength by protein phosphatase 1. *Neuron* **32**, 1133–1148 doi:10.1016/S0896-6273(01)00554-2

38 Mulkey, R.M., Endo, S., Shenolikar, S. and Malenka, R.C. (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* **369**, 486–488 doi:10.1038/369486a0

39 Snyder, G.L., Galdi, S., Fienberg, A.A., Allen, P., Nairn, A.C. and Greengard, P. (2003) Regulation of AMPA receptor dephosphorylation by glutamate receptor agonists. *Neuropharmacology* **45**, 703–713 doi:10.1016/S0028-3908(03)00319-8

40 Beattie, E.C., Carroll, R.C., Yu, X., Morishita, W., Yasuda, H., von Zastrow, M. et al. (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat. Neurosci.* **3**, 1291–1300 doi:10.1038/81823

41 Snyder, E.M., Colledge, M., Crozier, R.A., Chen, W.S., Scott, J.D. and Bear, M.F. (2005) Role for A kinase-anchoring proteins (AKAPS) in glutamate receptor trafficking and long term synaptic depression. *J. Biol. Chem.* **280**, 16962–16968 doi:10.1074/jbc.M409693200

42 Sanderson, J.L., Gorski, J.A. and Dell'Acqua, M.L. (2016) NMDA receptor-dependent LTD requires transient synaptic incorporation of Ca^{2+}-permeable AMPARs mediated by AKAP150-anchored PKA and calcineurin. *Neuron* **89**, 1000–1015 doi:10.1016/j.neuron.2016.01.043

43 Hell, J.W. (2016) How Ca^{2+}-permeable AMPA receptors, the kinase PKA, and the phosphatase PP2B are intertwined in synaptic LTP and LTD. *Sci. Signal.* **9**, pe2 doi:10.1126/scisignal.aaf7067

44 Brandon, N.J., Jovanovic, J.N., Colledge, M., Kittler, J.T., Brandon, J.M., Scott, J.D. et al. (2003) A-kinase anchoring protein 79/150 facilitates the phosphorylation of GABA(A) receptors by cAMP-dependent protein kinase via selective interaction with receptor β subunits. *Mol. Cell. Neurosci.* **22**, 87–97 doi:10.1016/S1044-7431(02)00017-9

45 Mercado, J., Baylie, R., Navedo, M.F., Yuan, C., Scott, J.D., Nelson, M.T. et al. (2014) Local control of TRPV4 channels by AKAP150-targeted PKC in arterial smooth muscle. *J. Gen. Physiol.* **143**, 559–575 doi:10.1085/jgp.201311050

46 Nystoriak, M.A., Nieves-Cintron, M., Nygren, P.J., Hinke, S.A., Nichols, C.B., Chen, C.-Y. et al. (2014) AKAP150 contributes to enhanced vascular tone by facilitating large-conductance Ca^{2+}-activated K^+ channel remodeling in hyperglycemia and diabetes mellitus. *Circ. Res.* **114**, 607–615 doi:10.1161/CIRCRESAHA.114.302168

47 Cheng, E.P., Yuan, C., Navedo, M.F., Dixon, R.E., Nieves-Cintron, M., Scott, J.D. et al. (2011) Restoration of normal L-type Ca^{2+} channel function during Timothy syndrome by ablation of an anchoring protein. *Circ. Res.* **109**, 255–261 doi:10.1161/CIRCRESAHA.111.248252

48 Nichols, C.B., Rossow, C.F., Navedo, M.F., Westenbroek, R.E., Catterall, W.A., Santana, L.F. et al. (2010) Sympathetic stimulation of adult cardiomyocytes requires association of AKAP5 with a subpopulation of L-type calcium channels. *Circ. Res.* **107**, 747–756 doi:10.1161/CIRCRESAHA.109.216127

49 Hinke, S.A., Navedo, M.F., Ulman, A., Whiting, J.L., Nygren, P.J., Tian, G. et al. (2012) Anchored phosphatases modulate glucose homeostasis. *EMBO J.* **31**, 3991–4004 doi:10.1038/emboj.2012.244

50 Sanderson, J.L. and Dell'Acqua, M.L. (2011) AKAP signaling complexes in regulation of excitatory synaptic plasticity. *Neuroscientist.* **17**, 321–336 doi:10.1177/1073858410384740

51 Oliveria, S.F., Gomez, L.L. and Dell'Acqua, M.L. (2003) Imaging kinase–AKAP79–phosphatase scaffold complexes at the plasma membrane in living cells using FRET microscopy. *J. Cell. Biol.* **160**, 101–112 doi:10.1083/jcb.200209127

52 Swope, S.L., Moss, S.J., Raymond, L.A. and Huganir, R.L. (1999) Regulation of ligand-gated ion channels by protein phosphorylation. *Adv. Second Messenger Phosphoprotein Res.* **33**, 49–78 doi:10.1016/S1040-7952(99)80005-6

53 McCaffrey, P.G., Luo, C., Kerppola, T.K., Jain, J., Badalian, T.M., Ho, A.M. et al. (1993) Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. *Science* **262**, 750–754 doi:10.1126/science.8235597
54 Haddy, A., Swanson, S.K.-H., Born, T.L. and Rusnak, F. (1992) Inhibition of calcineurin by cyclosporin A-cyclophilin requires calcineurin B. *FEBS Lett.* **314**, 37–40 doi:10.1016/0014-5793(92)81456-V
55 Liu, J., Farmer, J.D.J., Lane, W.L., Friedman, J., Weissman, I. and Schreiber, S.L. (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* **66**, 807–815 doi:10.1016/0092-8674(91)90124-H
56 Li, J., Negro, A., Lopez, J., Bauman, A.L., Henson, E., Dodge-Kafka, K. et al. (2010) The mAKAPβ scaffold regulates cardiac myocyte hypertrophy via recruitment of activated calcineurin. *J. Mol. Cell. Cardiol.* **48**, 387–394 doi:10.1016/j.yjmcc.2009.10.023
57 Santana, L.F., Chase, E.G., Votaw, V.S., Nelson, M.T. and Greven, R. (2002) Functional coupling of calcineurin and protein kinase A in mouse ventricular myocytes. *J. Physiol.* **544**, 57–69 doi:10.1113/jphysiol.2002.020552
58 De Windt, L.J., Lim, H.W., Bueno, O.F., Liang, Q., Delling, U., Braz, J.C. et al. (2001) Targeted inhibition of calcineurin attenuates cardiac hypertrophy *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 3322–3327. doi:10.1073/pnas.031371998
59 Kang, S., Li, H., Rao, A. and Hogan, P.G. (2005) Inhibition of the calcineurin-NFAT interaction by small organic molecules reflects binding at an allosteric site. *J. Biol. Chem.* **280**, 37698–37706 doi:10.1074/jbc.M502247200
60 Yu, H., van Berkel, T.J.C. and Biessen, E.A.L. (2007) Therapeutic potential of VIVIT, a selective peptide inhibitor of nuclear factor of activated T cells, in cardiovascular disorders. *Cardiovasc. Drug Rev.* **25**, 175–187 doi:10.1111/j.1527-3466.2007.00011.x
61 Lai, M.M., Burnett, P.E., Wolosker, H., Blackshaw, S. and Snyder, S.H. (1998) Cain, a novel physiologic protein inhibitor of calcineurin. *J. Biol. Chem.* **273**, 18325–18331. doi:10.1074/jbc.273.29.18325
62 Wesselborg, S., Fruman, D.A., Sagoo, J.K., Bierer, B.E. and Burakoff, S.J. (1996) Identification of a physical interaction between calcineurin and nuclear factor of activated T cells (NFATp). *J. Biol. Chem.* **271**, 1274–1277 doi:10.1074/jbc.271.3.1274
63 Czirják, G. and Enyedi, P. (2014) The LQLP calcineurin docking site is a major determinant of the calcium-dependent activation of human TRESK background K^+ channel. *J. Biol. Chem.* **289**, 29506–29518 doi:10.1074/jbc.M114.577684
64 Czirjak, G. and Enyedi, P. (2006) Targeting of calcineurin to an NFAT-like docking site is required for the calcium-dependent activation of the background K^+ channel, TRESK. *J. Biol. Chem.* **281**, 14677–14682 doi:10.1074/jbc.M602495200
65 Slupe, A.M., Merrill, R.A., Flippo, K.H., Lobas, M.A., Houtman, J.C.D. and Strack, S. (2013) A calcineurin docking motif (LXVP) in dynamin-related protein 1 contributes to mitochondrial fragmentation and ischemic neuronal injury. *J. Biol. Chem.* **288**, 12353–12365 doi:10.1074/jbc.M113.459677
66 Kolozsvari, B., Bako, E., Becsi, B., Kiss, A., Czikora, A., Toth, A. et al. (2012) Calcineurin regulates endothelial barrier function by interaction with and dephosphorylation of myosin phosphatase. *Cardiovasc. Res.* **96**, 494–503 doi:10.1093/cvr/cvs255
67 Bodmer, D., Ascaño, M. and Kuruvilla, R. (2011) Isoform-specific dephosphorylation of dynamin1 by calcineurin couples neurotrophin receptor endocytosis to axonal growth. *Neuron* **70**, 1085–1099 doi:10.1016/j.neuron.2011.04.025