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The interaction of assembly protein AP180 and clathrin is inhibited by multi-site phospho-mimetics

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ABSTRACT

Clathrin-mediated endocytosis at the nerve terminal is dependent on assembly protein 180 (AP180) and adapter protein complex 2 (AP2). Both membrane adapter proteins bind to each other and to clathrin, to drive assembly of the clathrin coat over nascent synaptic vesicles. Using knowledge of in vivo phosphorylation sites, AP180 was mutated to determine the effect on binding. N-terminally truncated AP180 exhibited phospho-mimetic (Ser/Thr to Glu)-dependent interaction with AP2, but not clathrin. C-terminally truncated and full length phospho-mutant AP180 bound less AP2 than wild type. However, there was no difference in AP2 binding for the phospho-mimetic or phospho-deficient (Ser/Thr to Ala) AP180 mutants. Thus, the phospho-mutant approach did not provide clarity for the role of phosphorylation in AP180-AP2 binding. Clathrin exhibited a phospho-mimetic-dependent interaction with full-length AP180. Furthermore, phospho-mimetic AP180 was deficient at assembling clathrin cages. These latter discoveries support a model where AP180 phosphorylation inhibits clathrin binding and assembly.

1. Introduction

Synaptic vesicle endocytosis (SVE) uses brain-specific homologs of the ubiquitous clathrin-mediated endocytosis protein machinery to form a clathrin coat around vesicles. The coat is tightly size-regulated for the quantal release of neurotransmitter (Moshkanbaryans et al., 2014; Poudel and Bai, 2014; Ye and Lafer, 1995). Assembly protein 180 (AP180) and adaptor protein complex 2 (AP2) drive formation of clathrin-coated pits (Hao et al., 1999). Both adapter proteins sort membrane proteins, required for exocytosis, into nascent synaptic vesicles (Harel et al., 2008; Koo et al., 2011). Thus, AP180 and AP2 ensure both synaptic vesicle release competency and quantal neurotransmitter release. Genetic deletion of AP180 in mice resulted in synaptic vesicle defects, excitatory/inhibitory imbalance and seizures (Koo et al., 2015).

AP180 has readily measurable turnover of phosphate, as demonstrated by multiple studies (Cousin et al., 2001; Keen and Black, 1986; Tan et al., 2003) and is dephosphorylated by calcineurin in response to KCl depolarisation of nerve terminals (Cousin and Robinson, 2001; Cousin et al., 2001). However, the function of AP180 phosphorylation is not clear. The prevailing model is that the main functions of AP180 are inhibited until activity-dependent signalling promotes dephosphorylation, thereby removing inhibition (Cousin and Robinson, 2001). AP180 was found to be an in vitro substrate of casein kinase 2 and this phosphorylation inhibited AP2 binding and cooperative clathrin assembly (Hao et al., 1999). The same study narrowed the location of the in vitro phosphorylation to a large central region of the clathrin and AP2 binding (CLAP) domain, which also contained a region of strongest AP2 binding (Fig. 1A), but the sites of phosphorylation were not identified.

KCl depolarisation of synaptosomes combined with phosphoproteomics analysis revealed down-regulation of phosphorylation (dephosphorylation) at S600, S621 and S627, specific to the isoform 2 of AP180 (Kohansal-Nodehi et al., 2016). The longer isoform 1 contains an amino acid sequence not conserved in humans or mice. More recently, S296, S300, S306 and S313 were also found to be down-regulated following depolarisation in synaptosomes (Engholm-Keller et al., 2019). The same study reported AP180 phosphorylation sites from cultured hippocampal neurons that were up-regulated (S16 and S767) or down-regulated (S305, T310, T312, S313, S600 [short] and S627 [short]) following depolarisation. Greater than thirty AP180 phosphorylation sites exist in on-line repositories (Hornbeck et al., 2015), including O-linked N-acetylglucosamine-6-phosphorylation (Graham et al., 2011).

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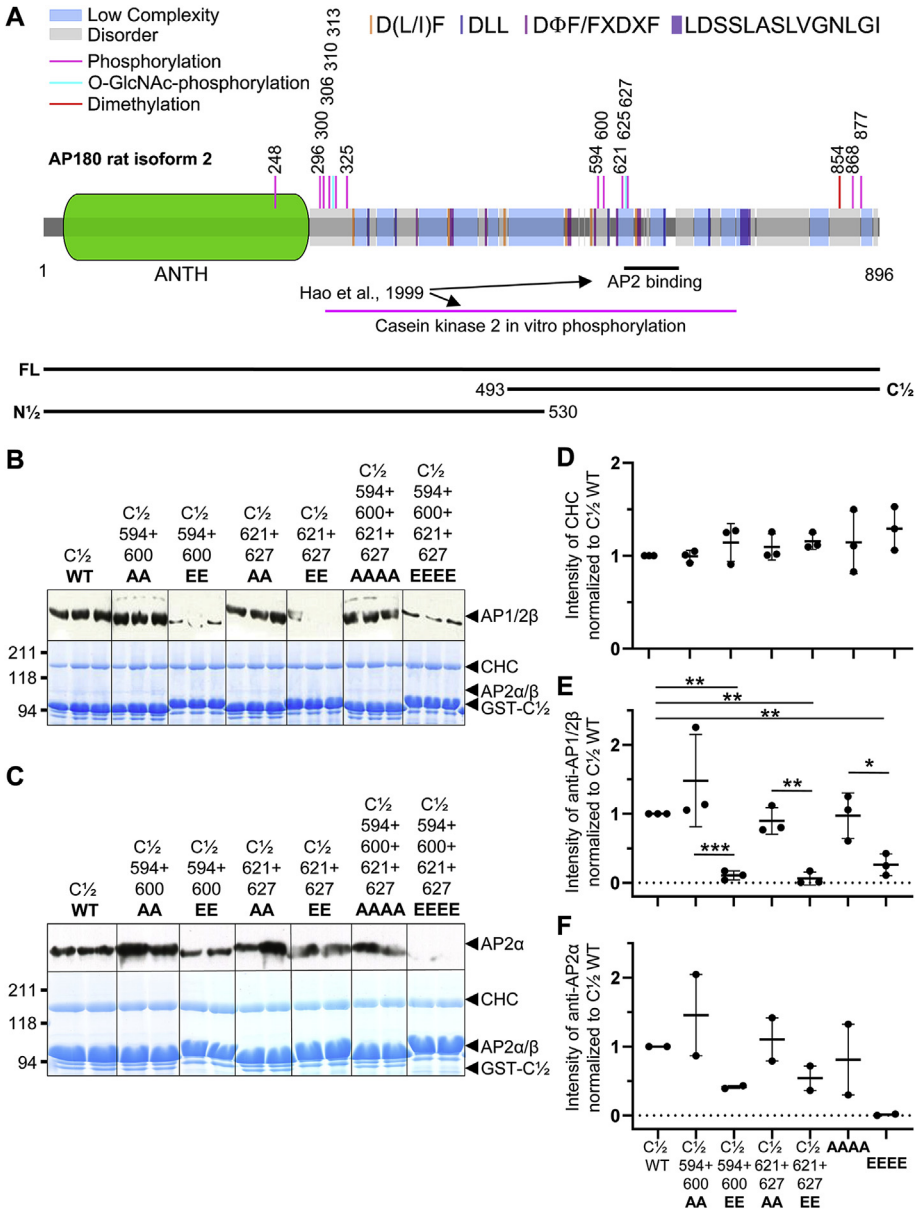

Fig. 1. AP2 binding to the C-terminal half of AP180 is inhibited by phosphorylation site mutation. (A) The in vivo phosphorylation sites of AP180 from synaptosomes determined by LC-MS/MS displayed on the AP180 domain structure, including AP2 and clathrin binding motifs, low complexity and disordered regions. The strong AP2 binding (623–680) and in vitro phosphorylation regions (305–744) identified previously (Hao et al., 1999) are indicated. Below, are the AP180 sequences used in this work. The amount of bound AP2 from a pull-down from synaptosome lysate with GST-tagged C½ WT and phospho-mutants is shown by Western blot detection with (B) anti-AP1/2β and (C) anti-AP2α. An SDS-PAGE gel was stained with Coomassie Blue to show the amount of clathrin heavy chain (CHC) pulled-down and comparison of protein levels (loaded at 10% (B) and 40% (C) of blot protein level). (D) shows the densitometry of the Coomassie bands for CHC in (B). (E) and (F) show the densitometry for anti-AP1/2β and anti-AP2α in (B) and (C), respectively. (D–E) share the same x-axis labels shown for (F). For densitometry (D–F), the intensity of each band was divided by the intensity of the Coomassie C½ bands. The data is expressed as average intensity of detection for CHC ± SD (n = 3), anti-AP1/2β ± SD (n = 3) and anti-AP2α ± the range (n = 2), as a fraction of the result for C½ WT. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Some of the phosphorylation sites are interspersed with multiple short binding motifs within the AP180 CLAP domain, which have been identified as mediating interaction with AP2 (Brett et al., 2002; Mishra et al., 2004; Praefcke et al., 2004) and clathrin (Morgan et al., 2000; Moshkanbaryans et al., 2016; ter Haar et al., 2000; Zhuo et al., 2010), but no sites are associated with phospho-dependent functions.

We screened for AP180 phosphorylation sites from synaptosomes and investigated how mutation of different groups of phosphorylation sites affected AP2 and clathrin binding using N- and C-terminally truncated AP180, as well as the full length AP180. Binding of AP2 was consistently, but not specifically, inhibited by phospho-mimetic mutation. However, there was clear evidence for specific phospho-mimetic inhibition of the full length AP180 interaction with clathrin.

2. Materials and methods

2.1. Antibodies

Anti-Clathrin [X22] was from Abcam (#ab2731). Anti-AP2α (#610502, clone: 8/Adaptin α) and anti-adaptin β (#610382, clone: 74/Adaptin β) were from BD Transduction Laboratories. Primary antibodies were used at 1:1000 and horseradish peroxidase-conjugated secondary antibodies (DAKO) at 1:20,000.

2.2. Plasmids

The glutathione S-transferase (GST)-fusions used for Fig. 1B were based on the pGEX-6P-1 vector containing the DNA for mouse AP180 isoform 2 (equal in length and 98% identical to rat isoform 2) and was described previously (Chan et al., 2014). A codon optimised AP180 DNA sequence for improved bacterial expression (Chan et al., 2014) was used to produce all other GST-fusions (Fig. 2A–F).

2.3. Identification of AP180 phosphorylation sites by mass spectrometry

AP180 was purified from rat brain synaptosomes and analysed for phosphorylation by mass spectrometry, as described previously (Graham et al., 2011). Experiments involving rats were approved by the Children's Medical Research Institute/Children's Hospital Westmead Animal Ethics Committee. Further details of the mass spectrometry analysis are provided in Supplementary Material.

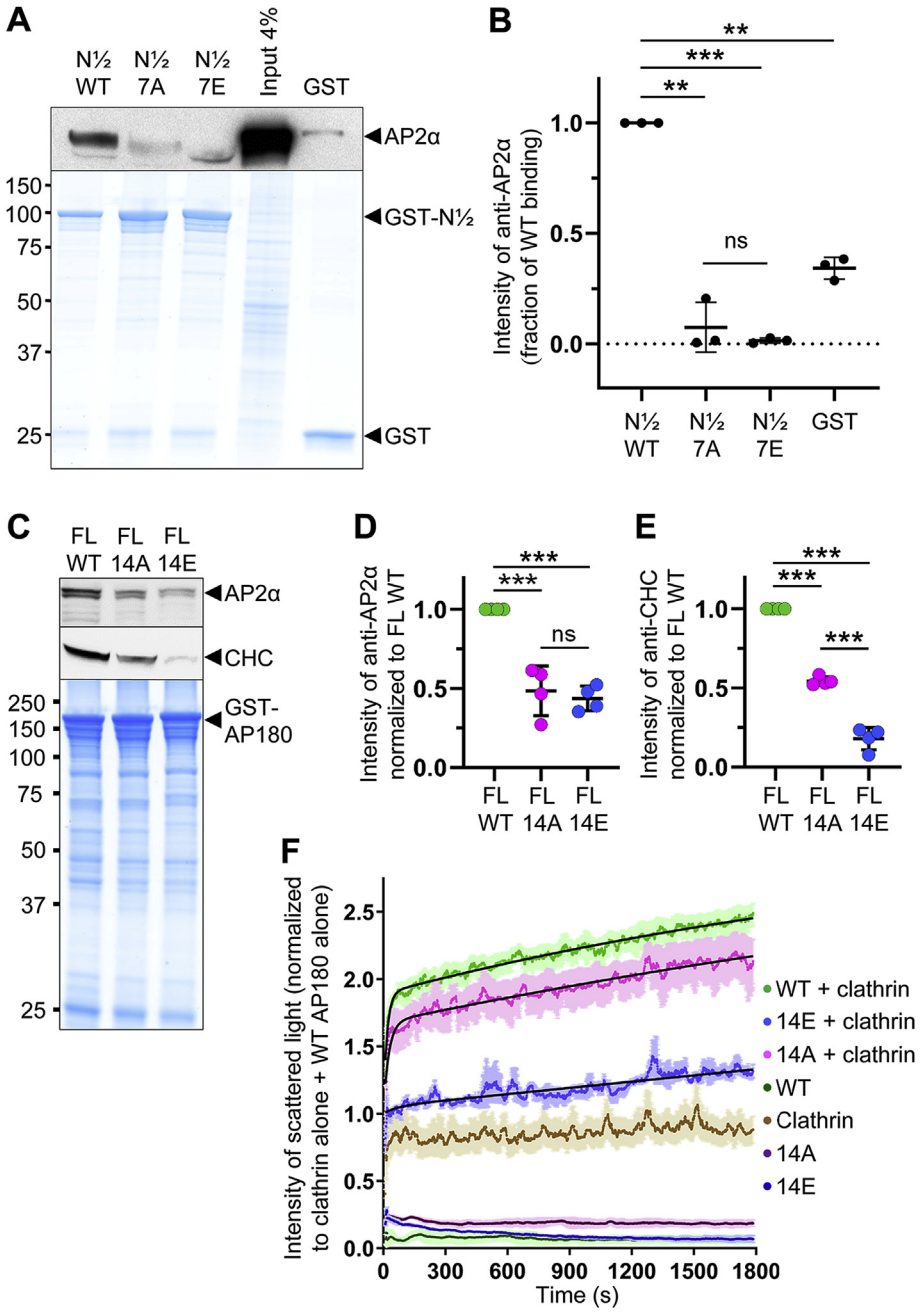

Fig. 2. Phospho-mimetic mutations affect binding to the N-terminal half of AP180 and both binding and assembly properties of full-length AP180. (A) Binding of AP2 to GST, GST-N½ WT and phospho-mutants (7A/E) detected by Western blot with anti-AP2α. A Coomassie Blue stained SDS-PAGE gel is shown for comparison of GST-fusion protein levels (loaded at 10% of the Western blot protein level). (B) Densitometry for the experiment in (A) (n = 3). The intensity of Western blot bands was divided by the intensity of the corresponding Coomassie Blue bands. **, $P < 0.01$; ***, $P < 0.001$. (C) Western blot detection with anti-AP2α and anti-CHC following a pull-down using GST-tagged AP180 FL WT, 14A and 14E. An SDS-PAGE gel was stained with Coomassie Blue for comparison of protein levels (loaded at 10% of blot protein level). (D) and (E) show the densitometry for anti-AP2α and anti-CHC in (C), respectively. Data is expressed as average amount of protein bound as a fraction of the WT pull-down ± SD (n = 4). 'ns' = not significant. (F) Effect of phosphorylation site mutation on clathrin assembly using AP180 FL WT, 14A and 14E, with the GST tag removed. The intensity of light scattering (y-axis) was normalised to the sum of intensities for clathrin alone and AP180 alone. The intensities shown are the mean ± SEM (n = 3) and a fitted curve is shown in black. The extra sum-of-squares F test (GraphPad Prism 8) was used to determine that the 14A and 14E curves were significantly different to WT ($P = 0.0002$ and $P < 0.0001$, respectively).

2.4. Binding studies using GST-fusions of AP180

GST-AP180 was expressed in bacteria as described previously (Chan et al., 2014). The preparation of rat brain synaptosomes, lysis, pull-down experiments, SDS-PAGE and western blotting were performed as described previously (Chan et al., 2014). Enhanced chemiluminescent reagent (ThermoFisher Scientific) was detected by an ImageQuant LAS 4000 digital reader (GE Life Sciences). The statistical significance of the SDS-PAGE and western blotting was determined by a one-way analysis of variance with correction for multiple comparisons using the method of Benjamini, Krieger and Yekutieli with a false discovery rate of 5% using GraphPad Prism 8.

2.5. Clathrin assembly assay

The components for the clathrin assembly assay, including 0.5 μM of each protein, were combined as described previously (Moshkanbaryans et al., 2016) and scattered light was detected using a fluorometer (LS50B Fluorescence Spectrometer, PerkinElmer) by setting excitation and emission wavelengths at 395 nm. Slit width was 2.5 nm, cuvette volume was 100 μl and intensity was recorded at 1 Hz. The data was curve fitted using GraphPad Prism 8 to determine the initial assembly rate, as described previously (Moshkanbaryans et al., 2016).

3. Results

3.1. The detection of AP180 phosphorylation sites from synaptosomes

AP180 was enriched by pull-down with GST-AP2α ear domain from rat brain synaptosome lysate (Engholm-Keller et al., 2018; Tan et al., 2003), purified by SDS-PAGE and digested with trypsin. Phosphopeptides were enriched and analysed by LC-MS/MS. Two of the detected sites were novel, S868 and S877 (isoform 2 numbering, Fig. S1B-C). These two sites were detected within a peptide that was also

dimethylated at R854 (Fig. 1A, Fig. S1). Supplementary Table S1 contains detailed information on the identification of ten sites (S248, S296, S300, S313, S325, S594, S600, S621, S868 and S877). We previously identified S306, S313, S621 and S627 phosphorylation sites, the O-GlcNAc-phosphorylation site T310 and a putative site at T625 using an identical purification method (Graham et al., 2011).

Our previous work indicates that the GST-AP2α ear domain does not discriminate between phosphorylated and non-phosphorylated AP180, although we cannot rule this out completely (Engholm-Keller et al., 2018; Tan et al., 2003). For subsequent experiments, we ignored AP180 phosphorylation sites detected by others that were either not from synaptosomes, not known to us at the time or had a relatively low number of independent detections (Hornbeck et al., 2015). Thus, we limited our binding and functional analysis to the fourteen AP180 phosphorylation sites shown in Fig. 1A.

3.2. The central group of phospho-mimetic mutations in N-terminally truncated AP180 inhibits AP2 and not clathrin binding

Strongest AP2 binding was previously localised to a region of AP180 (Hao et al., 1999) adjacent to the centrally located phosphorylation sites we identified (Fig. 1A). S594, S600, S621 and S627 were mutated to A ('phospho-deficient') or E ('phospho-mimetic') as pairs (AA/EE) or as a group of four (AAAA/EEEE) in the C½ sequence. Western blot detection of AP2α and AP1/2β was used to indicate binding of AP2 to C½ WT and phospho-mutants (Fig. 1B and C). Coomassie blue detection of the clathrin heavy chain (CHC) indicated that clathrin binding to C½ and phospho-mutant C½ was not significantly different (Fig. 1B–D). The EE and EEEE phospho-mutants were effective in nearly abolishing AP1/2 binding (Fig. 1E and F). The anti-AP1/2β was not specific enough to indicate AP2 binding, since it detects both β adaptins; however, there was good agreement between the AP2 complex-specific anti-AP2α (n = 2) and non-specific anti-AP1/2β (n = 3) results. Thus, phospho-mimetic mutation of the central group of sites inhibited AP2, but not clathrin binding to C½.

3.3. The N-terminal group of phospho-mimetic mutations inhibit AP2 binding in C-terminally truncated AP180

We showed previously that clathrin does not bind the first half of AP180 (Chan et al., 2014), N½ in Fig. 1A. N½ contains activity-dependent phosphorylation sites, but there has been no previous investigation of AP2 binding to N½. N½ also overlaps with a region of in vitro phosphorylation that influenced AP2 binding (Hao et al., 1999) (Fig. 1A). We determined whether the group of seven AP180 phosphorylation sites (S248, S296, S300, S306, T310, S313 and S325), mutated in N½ (Fig. 1A), could affect AP2 binding in pull-down experiments with synaptosome lysate. Western blot detection of the amount of AP2α extracted by GST-tagged N½ WT was low compared to the input, but significantly more than that extracted by GST alone (Fig. 2A and B). A comparison of N½ WT to 7A and 7E revealed that binding of AP2 to both the mutants was significantly less than the N½ WT (Fig. 2A and B). Since there was no significant difference between 7A and 7E, the interpretation of how phosphorylation might affect AP2 binding is ambiguous.

3.4. Mutating all detected phosphorylation sites in full length AP180 affects both AP2 and clathrin binding

Since we detected phosphorylation at multiple sites along the length of AP180, we tested whether these sites could work in concert to change the binding properties of AP180. All 14 of our curated set of synaptosome in vivo phosphorylation sites were mutated to A (14A) or E (14E) and used in a pull-down experiment. AP2α and CHC were detected by Western blot (Fig. 2C). AP2 binding to both AP180 14A and 14E was significantly different to WT (Fig. 2D). However, AP2 binding to 14A was not significantly different to 14E. Both the 14A and 14E mutant caused significantly different binding of clathrin compared to the FL WT AP180 (Fig. 2E). Clathrin binding to 14A was also significantly different to 14E and more pronounced than the loss of AP2 binding, demonstrating that mutation of AP180 phosphorylation sites affected clathrin binding.

3.5. Multi-site AP180 phospho-mimetic mutation inhibits clathrin assembly

The phospho-mimetic AP180-clathrin interaction was tested functionally using a clathrin assembly assay. Purified clathrin was mixed with FL WT AP180, 14A or 14 E phospho-mutants, which had the GST-tag removed (Fig. S2). Clathrin cage assembly was measured by light scattering (Fig. 2F). Clathrin can weakly self-assemble. The amount of light scattered by clathrin alone and AP180 alone was summed to form the experimental baseline. The initial rate of assembly of clathrin cages was calculated, since it was the main factor separating the curves. The rates were $0.578 \pm 0.025\,ms^{-1}$, $0.388 \pm 0.031\,ms^{-1}$ and $0.142 \pm 0.053\,ms^{-1}$ for FL WT, 14A and 14E, respectively. There was an approximately 30% lower initial rate for 14A, compared to WT. However, the largest effect was the approximately 4-fold reduction in the initial assembly rate for the 14E mutant compared to WT. The data demonstrates that phospho-mimetic mutation severely inhibits the ability of AP180 to assemble clathrin.

4. Discussion

Previously, AP2 binding was thought to occur at a single region of AP180 mediated by unidentified in vitro phosphorylation sites (Fig. 1A). These same in vitro sites affected AP180-AP2 cooperative clathrin assembly, but not assembly by phosphorylated AP180 alone (Hao et al., 1999). We mutated groups of AP180 phosphorylation sites that occur in vivo, some of which have been shown to undergo activity-dependent dephosphorylation. We demonstrate for the first time that phospho-mimetic substitution of AP180 phosphorylation sites affects clathrin binding and assembly, and that specific site substitutions affect AP2 binding.

Combinations of E, but not A, mutations of S594, S600, S621 and S627 in the C-terminal half of AP180 severely reduced AP2 binding. However, both A and E mutations in the N-terminal half and FL AP180 reduced AP2 binding. Loss of binding following S/T to A mutation is open to interpretation. Mutation could have caused a change in AP180 structure that prevents binding and is either (i) independent of the potential effect of phosphorylation or (ii) representative of the potential effect of phosphorylation on a structurally vulnerable region. Although AP180 C-terminal binding of AP2 was dependent on phospho-deficient mutations, our results are ambiguous as to whether full length AP180-AP2 binding might be phospho-regulated. One way to resolve this ambiguity might be by performing in vitro binding studies using phosphorylated AP180. However, there is currently no definitive evidence for the identity of the protein kinases responsible for phosphorylating AP180.

The 14E mutation in FL AP180 was very effective in preventing clathrin binding. Since there was no hint of phospho-mimetic-regulation of clathrin binding using the C-terminal half of AP180, we propose that the entirety of the AP180 assembly domain (~285–896, Fig. 1A) is utilised in its interaction with clathrin and failure to block most clathrin binding sub-regions via multi-site phosphorylation would fail to strongly inhibit clathrin binding. There are many clathrin binding motifs within low complexity regions of AP180 interspersed with disordered regions that are potentially targeted by protein kinases (Iakoucheva et al., 2004) (Fig. 1A). Given the phosphorylation site knowledge stored within databases (Hornbeck et al., 2015) and the lack of access to large regions of AP180 using trypsin (Moshkanbaryans et al., 2014), it is likely that the sites we have focused on here are not the full complement of phosphorylation sites regulating AP180 binding.

The multi-phosphorylation site mutation strategy used here may be a general approach for determining whether interactions by large regions of low complexity are regulated by phosphorylation and could be applied to other SVE adapter proteins. In conclusion, this work supports a model where the function of phospho-signalling directed at AP180 is to regulate clathrin binding and assembly in SVE.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104474.

References

Brett, T.J., Traub, L.M., Fremont, D.H., 2002. Accessory protein recruitment motifs in clathrin-mediated endocytosis. Structure 10, 797–809.
Chan, L.S., Moshkanbaryans, L., Xue, J., Graham, M.E., 2014. The approximately 16 kDa C-terminal sequence of clathrin assembly protein AP180 is essential for efficient clathrin binding. PLoS One 9, e110557.
Cousin, M.A., Robinson, P.J., 2001. The dephosphins: dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. Trends Neurosci. 24, 659–665.
Cousin, M.A., Tan, T.C., Robinson, P.J., 2001. Protein phosphorylation is required for endocytosis in nerve terminals: potential role for the dephosphins dynamin I and synaptojanin, but not AP180 or amphiphysin. J. Neurochem. 76, 105–116.
Engholm-Keller, K., Bache, N., Rao, S.R., Wark, J.R., Larsen, M.R., Robinson, P.J., Graham, M.E., 2018. In: Murphy, K.M. (Ed.), Affinity Proteomics for Interactome and Phosphoproteome Screening in Synaptosomes. Synaptosomes,Springer New York, New York, NY, pp. 165–191.
Engholm-Keller, K., Waardenberg, A.J., Muller, J.A., Wark, J.R., Fernando, R.N., Arthur, J.W., Robinson, P.J., Dietrich, D., Schoch, S., Graham, M.E., 2019. The temporal profile of activity-dependent presynaptic phospho-signalling reveals long-lasting patterns of poststimulus regulation. PLoS Biol. 17, e3000170.
Graham, M.E., Thaysen-Andersen, M., Bache, N., Craft, G.E., Larsen, M.R., Packer, N.H., Robinson, P.J., 2011. A novel post-translational modification in nerve terminals: O-linked N-acetylglucosamine phosphorylation. J. Proteome Res. 10, 2725–2733.
Hao, W., Luo, Z., Zheng, L., Prasad, K., Lafer, E.M., 1999. AP180 and AP-2 interact directly in a complex that cooperatively assembles clathrin. J. Biol. Chem. 274, 22785–22794.
Harel, A., Wu, F., Mattson, M.P., Morris, C.M., Yao, P.J., 2008. Evidence for CALM in directing VAMP2 trafficking. Traffic 9, 417–429.
Hornbeck, P.V., Zhang, B., Murray, B., Kornhauser, J.M., Latham, V., Skrzypek, E., 2015. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res. 43, D512–D520.
Iakoucheva, L.M., Radivojac, P., Brown, C.J., O'Connor, T.R., Sikes, J.G., Obradovic, Z., Dunker, A.K., 2004. The importance of intrinsic disorder for protein phosphorylation. Nucleic Acids Res. 32, 1037–1049.
Keen, J.H., Black, M.M., 1986. The phosphorylation of coated membrane proteins in intact neurons. J. Cell Biol. 102, 1325–1333.
Kohansal-Nodehi, M., Chua, J.J., Urlaub, H., Jahn, R., Czernik, D., 2016. Analysis of protein phosphorylation in nerve terminal reveals extensive changes in active zone proteins upon exocytosis. Elife 5.
Koo, S.J., Kochlamazashvili, G., Rost, B., Puchkov, D., Gimber, N., Lehmann, M., Tadeus, G., Schmoranzer, J., Rosenmund, C., Haucke, V., Maritzen, T., 2015. Vesicular synaptobrevin/VAMP2 levels guarded by AP180 control efficient neurotransmission. Neuron 88, 330–344.
Koo, S.J., Markovic, S., Puchkov, D., Mahrenholz, C.C., Beceren-Braun, F., Maritzen, T., Dernedde, J., Volkmer, R., Oschkinat, H., Haucke, V., 2011. SNARE motif-mediated sorting of synaptobrevin by the endocytic adaptors clathrin assembly lymphoid myeloid leukemia (CALM) and AP180 at synapses. Proc. Natl. Acad. Sci. U. S. A. 108, 13540–13545.
Mishra, S.K., Hawryluk, M.J., Brett, T.J., Keyel, P.A., Dupin, A.L., Jha, A., Heuser, J.E., Fremont, D.H., Traub, L.M., 2004. Dual engagement regulation of protein interactions with the AP-2 adaptor alpha appendage. J. Biol. Chem. 279, 46191–46203.
Morgan, J.R., Prasad, K., Hao, W., Augustine, G.J., Lafer, E.M., 2000. A conserved clathrin assembly motif essential for synaptic vesicle endocytosis. J. Neurosci. 20, 8667–8676.
Moshkanbaryans, L., Chan, L.S., Graham, M.E., 2014. The biochemical properties and functions of CALM and AP180 in clathrin mediated endocytosis. Membranes 4, 388–413.
Moshkanbaryans, L., Xue, J., Wark, J.R., Robinson, P.J., Graham, M.E., 2016. A novel sequence in AP180 and CALM promotes efficient clathrin binding and assembly. PLoS One 11, e0162050.
Poudel, K.R., Bai, J., 2014. Synaptic vesicle morphology: a case of protein sorting? Curr. Opin. Cell Biol. 26, 28–33.
Praefcke, G.J., Ford, M.G., Schmid, E.M., Olesen, L.E., Gallop, J.L., Peak-Chew, S.Y., Vallis, Y., Babu, M.M., Mills, I.G., McMahon, H.T., 2004. Evolving nature of the AP2 alpha-appendage hub during clathrin-coated vesicle endocytosis. EMBO J. 23, 4371–4383.
Tan, T.C., Valova, V.A., Malladi, C.S., Graham, M.E., Berven, L.A., Jupp, O.J., Hansra, G., McClure, S.J., Sarcevic, B., Boadle, R.A., Larsen, M.R., Cousin, M.A., Robinson, P.J., 2003. Cdk5 is essential for synaptic vesicle endocytosis. Nat. Cell Biol. 5, 701–710.
ter Haar, E., Harrison, S.C., Kirchhausen, T., 2000. Peptide-in-groove interactions link target proteins to the beta-propeller of clathrin. Proc. Natl. Acad. Sci. U. S. A. 97, 1096–1100.
Ye, W., Lafer, E.M., 1995. Bacterially expressed F1-20/AP-3 assembles clathrin into cages with a narrow size distribution: implications for the regulation of quantal size during neurotransmission. J. Neurosci. Res. 41, 15–26.
Zhuo, Y., Ilangovan, U., Schirf, V., Demeler, B., Sousa, R., Hinck, A.P., Lafer, E.M., 2010. Dynamic interactions between clathrin and locally structured elements in a disordered protein mediate clathrin lattice assembly. J. Mol. Biol. 404, 274–290.