

Phosphatidylinositol Binding Clathrin-Assembly Protein and Alzheimer's Disease

Subjects: [Cell Biology](#)

Contributor: Kunie Ando , Siranjeevi Nagaraj , Fahri Küçükali , Marie-Ange de Fisenne , Andreea-Claudia Kosa , Emilie Doeraene , Lidia Lopez Gutierrez , Jean-Pierre Brion , Karelle Leroy

Genome-wide association studies (GWAS) have identified the *PICALM* (Phosphatidylinositol binding clathrin-assembly protein) gene as the most significant genetic susceptibility locus after *APOE* and *BIN1*. *PICALM* is a clathrin-adaptor protein that plays a critical role in clathrin-mediated endocytosis and autophagy.

PICALM

Alzheimer's disease

GWAS

amyloid β

1. Physiopathology of Alzheimer's Disease and Genetic Risk Factors

Alzheimer's disease (AD) is the most common cause of dementia, manifesting itself in cognitive deficits. AD is characterized by two neuropathological hallmarks: extracellular deposition of fibrils made up of amyloid β ($A\beta$) peptides as senile plaques [1] and intracellular accumulation of hyperphosphorylated tau as neurofibrillary tangles (NFTs) [2].

$A\beta$ is produced by successive cleavages of β -amyloid precursor protein (APP), encoded by *APP* gene located in chromosome 21, by β -secretase (BACE1) and γ -secretase [3]. APP is cleaved by BACE1 at its N terminal domain to release soluble APP- β and the membrane-bound APP C-terminal fragment (β -CTF or C99). The γ -secretase is a complex of four protein subunits consisting of presenilin (PSEN), Nicastrin, presenilin enhancer (PEN) and APH1 (anterior pharynx-defective 1). Sequential cleavages of APP by BACE1 and γ -secretase lead to production of $A\beta$ peptides of 38-, 40- and 42-amino acids in length. $A\beta$ peptides are principally produced in endosomes and are released from neurons in a synaptic activity-dependent manner [4]. $A\beta$ peptides adopt aggregation-prone conformations that are resistant to proteolysis and form oligomers, protofibrils and fibrils. Due to hydrophobicity at its C-terminus, $A\beta_{42}$ has greater aggregation properties [5].

Tau is a microtubule-associated protein and has a role in stabilizing neuronal microtubules. Tau is encoded by *MAPT* (microtubule-associated protein tau) gene located on chromosome 17. Tau is natively soluble and unfolded, but tau can be aggregated into oligomers and fibrils in the presence of pathologically misfolded tau or polyanions [6]. In AD, tau aggregates form paired helical filaments (PHF) or straight filaments [7]. Compelling evidence suggests that tau pathology, defined by the accumulation of hyperphosphorylated aggregated forms of tau, is strongly associated with neurodegeneration and cognitive impairment in AD [5][8]. Tau pathology propagates between neuroanatomically connected areas throughout the brain during the progression of AD. The stereotypical

aspect of tau pathology progression forms the basis of neuropathological Braak staging of tau pathology [9]. The stage of tau pathology better correlates with cognitive decline than A β load in AD patients [8]. Numerous studies have suggested that both synthetic and AD brain-derived tau fibrils can propagate in a prion-like manner by recruiting normal unfolded tau into pathological tau aggregates [10]. Tau aggregation can also be found in neurons and glia of other human neurological disorders, so-called tauopathies, such as frontotemporal lobar degeneration (FTLD) with tau pathology, progressive supranuclear palsy (PSP), Pick disease, corticobasal degeneration (CBD), etc. [11].

AD can be generally classified into two subgroups according to the age of onset. AD cases occurring earlier than age 65 are termed early-onset AD (EOAD), constituting less than 5% of all cases. Early-onset AD includes familial AD (FAD) inherited in an autosomal-dominant manner and can present at a very early age of onset and with a more rapid rate of disease progression, constituting 1–2% of AD cases. A number of FAD mutations have been identified in the genes of *APP*, *Presenilin1 (PSEN1)* and *Presenilin2 (PSEN2)* (γ -secretase component) [12].

Since age is the most important risk factor for AD [13], the great majority of cases, estimated at more than 95%, occur after age 65, constituting late-onset AD (LOAD). There was little knowledge of genetic risk factors for LOAD except for *Apolipoprotein (APOE)* risk allele *APOE4* till the breakthrough made by genome-wide association studies (GWAS) [14][15]. To date, GWAS have identified about 75 single nucleotide polymorphisms (SNPs), including *APOE4*, *BIN1*, *PICALM*, *CLU*, *CR1*, *ABCA7*, *TREM2*, *MS4A* and *APH1B*, with a significant association for LOAD susceptibility [16]. These LOAD-susceptibility genes are functionally implicated in multiple cellular processes such as synaptic function, lipid metabolism, inflammation, endocytosis, cytoskeletal transports, and tau and amyloid pathways in AD pathologies [17][18][19]. Nevertheless, the mechanisms by which the GWAS hit genes modulate the AD risk remain largely elusive and have been under active scrutiny in the field of functional genomics in the post GWAS era. The vast majority of these variants are located in the noncoding region [20], and such variants are supposed to exert phenotypic effects via the perturbation of transcriptional gene promoters and enhancers [21].

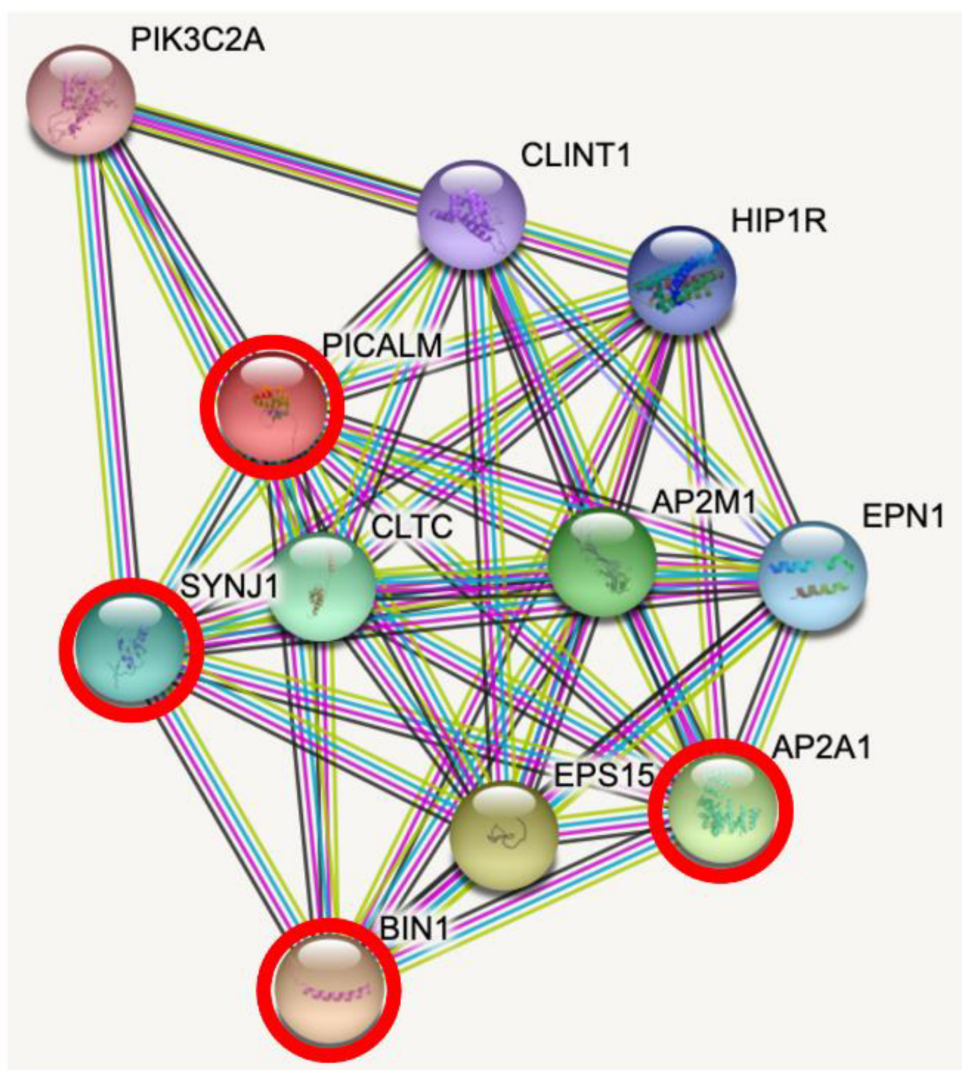
2. Biology of PICALM

2.1. Tissue Expression, Cellular Functions and Protein Interaction of PICALM

The human *PICALM* gene is located on chromosome 11 [22]. There are 24 transcripts as splice variants, 15 of them coding protein [22], whose sizes vary from 134 to 652 amino acids [23], and the molecular weights of the major isoforms are estimated at 65–75 kDa due to alternative splicing of exon 13 [24]. *PICALM* is a ubiquitous protein and is expressed in the brain, muscle, kidney, urinary bladder, connective tissues, bone marrow, lymphoid tissues and in female tissues, including breast and placenta, <https://www.proteinatlas.org/ENSG00000073921-PICALM/tissue> (accessed on 1 June 2022). [25]. In the human brain, *PICALM* is abundantly expressed in the microglia, oligodendrocytes, endothelial cells, neurons, vascular mural and choroid plexus cells (for further information on the localization of *PICALM* in the AD brain, see 3.3.1) [24][26][27][28][29][30]. In the mouse brain, *PICALM* mRNA is highly

expressed in the hippocampus [25], in particular in the dentate gyrus granule cells, as detected by a spatial transcriptomic study [31][32].

STRING database analysis revealed predicted protein–protein interactions of PICALM [33][34]. PICALM interacts with key proteins for clathrin-mediated endocytosis, intracellular trafficking and signaling (**Figure 1**). Interestingly, PICALM tightly interacts with other proteins encoded by GWAS hit genes for LOAD, such as *BIN1*, *CD2AP*, *EPHA1*, *AP2A1* and *AP2A2* [35]. PICALM is almost perfectly colocalized with the AP2 adaptor and AP2 α -adapting subunits (encoded by *AP2A1* and *AP2A2*) [36][37]. Interestingly, gene variants, splicing defects and altered expression of *AP2A1* and *AP2A2* have been recently shown to have a significant association with LOAD susceptibility [35]. Mounting evidence suggests PICALM has multiple roles in cellular functions and homeostasis as a clathrin adaptor for clathrin-mediated endocytosis, erythroid maturation, transferrin uptake, lipid homeostasis, autophagy, neuronal polarity, neuritic prolongation and synaptic vesicle turnover [38].




 LOAD susceptibility factor

Figure 1. PICALM interacts directly with several GWAS hits identified in LOAD. Predicted protein–protein interactions by STRING database analysis (<https://string-db.org> (accessed on 1 June 2022)) show several key proteins for clathrin-mediated endocytosis and intracellular trafficking as PICALM interactors [34]. *AP2A1* and *BIN1* have been identified as GWAS hits [35]. SNPs of *SYNJ1* have significant associations with disease onset of AD [39]. CLTC: Clathrin heavy chain 1. *SYNJ1*: Synaptojanin1. *PIK3C2A*: Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit α . *CLINT1*: Clathrin interactor 1. *HIP1R*: Huntingtin-interacting protein 1-related protein. *AP2M1*: AP2 complex subunit mu. *EPN1*: Epsin-1. *AP2A1*: AP2 complex subunit α -1. *EPS15*: Epidermal growth factor receptor substrate 15. *BIN1*: Myc box-dependent-interacting protein 1.

2.1.1. PICALM and Clathrin-Mediated Endocytosis

Clathrin-mediated endocytosis, also called receptor-mediated endocytosis, is a process leading to the internalization of plasma membrane-associated cargo molecules and subsequent trafficking through intracellular vesicle compartments. PICALM is necessary for clathrin assembly at the plasma membrane by binding to AP2 and clathrin (**Figure 2A**). PICALM interacts simultaneously with AP1 and clathrin at the trans-Golgi network in HeLa cells [37]. Several independent studies have reported that the depletion of PICALM leads to an increase in the size of clathrin-coated vesicles (CCVs) and to an increase in heterogeneity in the CCVs shape in cultured cells of rat hippocampus [40] and of HeLa cells [37][41]. CRISPR/Cas9-mediated PICALM disruption also leads to an increase in the number of early endosomes in HeLa cells [42]. A similar observation was obtained by the depletion of uncoordinated protein-11 (*unc-11*), the ortholog of PICALM in neurons of *C. elegans* [43]. Another study suggests that PICALM is required for efficient clathrin coat maturation in fibroblasts [44].

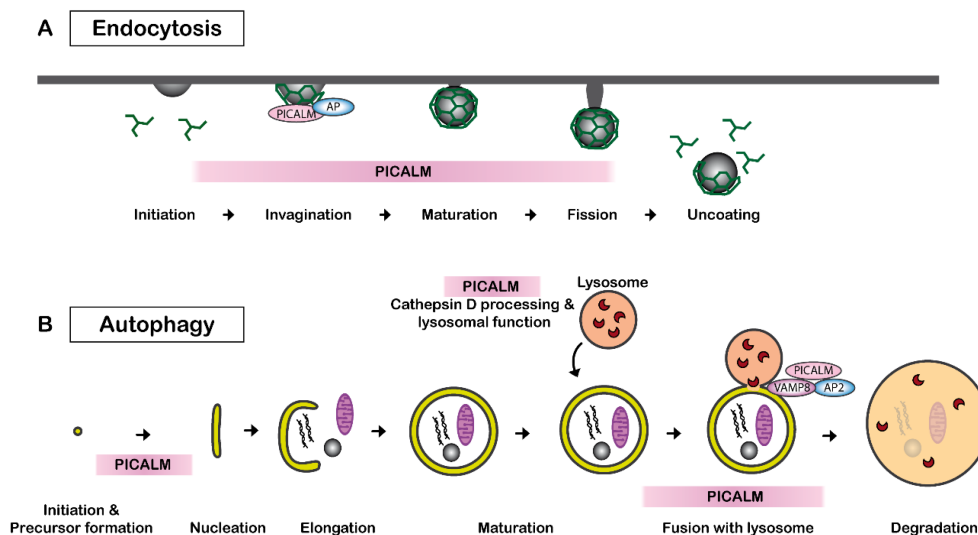


Figure 2. PICALM regulates clathrin-mediated endocytosis and autophagy. **(A)** PICALM is involved in clathrin-mediated endocytosis (CME) as a clathrin adaptor by facilitating the formation of clathrin-coated vesicles (CCVs). The interaction between PICALM, adaptor proteins (AP) and clathrin is critical to maintaining the form and sizes of CCVs. PICALM interacts with AP2 at the plasma membrane and AP 1 in the trans-Golgi network to form CCVs. **(B)** PICALM is also involved in multiple steps of autophagy process. PICALM is involved in the autophagy precursor formation at the autophagosome-lysosome fusion [45], maturation of Cathepsin D and lysosomal function [42].

2.1.2. PICALM, Hematopoiesis, Transferrin-Uptake and Cholesterol Homeostasis

Chromosomal translocations of *PICALM* have been observed in patients with acute lymphoblastic leukemia and acute myeloid leukemia [46]. In mice, nonsense point mutations of *PICALM* cause abnormal iron distribution, growth retardation, shortened life span and hematopoietic abnormalities [47].

PICALM is a critical protein, and homozygous *PICALM* knockout mice are dwarfed and die within 1 month after birth due to a deficiency in erythroid maturation and transferrin uptake [48]. There are controversies in the literature on the effects of *PICALM* on transferrin uptake. While some studies suggest that *PICALM* overexpression or down-expression did not alter transferrin uptake in HeLa cells or HEK293 cells [49][50], others observed a significant effect on transferrin uptake in the liver, bone marrow and erythroid cells from *PICALM*-deficient mice [44][48]. It is thus speculated that the deletion of *PICALM* may be partially compensated by other endocytic proteins in certain types of cells. Indeed, *PICALM* is dispensable in myeloid and B-lymphoid development [44].

Additionally, *PICALM* also modulates cellular cholesterol homeostasis, as *PICALM* expression influences the expression of genes encoding proteins involved in cholesterol biosynthesis and lipoprotein uptake [51]. Knockdown of *PICALM* leads to up to a 50% increase in cholesterol biosynthesis genes in HEK293, murine embryonic fibroblasts (MEF) and Cath-a-differentiated (CAD) neuronal cells [51].

2.1.3. The Roles of PICALM in Neuronal Polarity and Synaptic Vesicle Sorting

PICALM and its neuronal homolog assembly protein 180 (AP180) have quite interesting functions in neuronal cells. Endocytosis is reduced in *PICALM*- or AP180-deficient neurons [52]. Interestingly, *PICALM* and AP180 have critical roles in establishing the polarity and neuritic growth in neuronal cells. While treatments with siRNA targeting *PICALM* lead to dendritic dystrophy, treatments with siRNA targeting *AP180* abolish axonal extension in rat primary neuronal culture. In contrast, neurons overexpressing AP180 or *PICALM* generate multiple axons [52].

PICALM is expressed in neurons and is present in synapses [30]. *PICALM* regulates membrane fusion by binding to R-SNAREs (soluble NSF attachment protein receptors) such as VAMP2, VAMP3 and VAMP8 [53][54]. Furthermore, *PICALM* regulates the internalization of VAMP2 (Synaptobrevin 2), the most abundant synaptic vesicle protein and a major R-SNARE component [50]. Since the AP180 N-terminal homology (ANTH) domain (see chapter 2.2) of *PICALM* interacts with VAMP2, both *PICALM* and AP180 regulate endocytic sorting of synaptic vesicles at active zones for neurotransmission [53]. *PICALM* and related ANTH-domain-containing proteins modulate the surface levels of calcium-permeable AMPA-type glutamate receptors (AMPA receptors) that mediate fast excitatory neurotransmission and excitotoxicity [55]. *PICALM* is thereby supposed to play roles in synaptic plasticity and learning by modulating both long-term potentiation (LTP) and long-term depression (LTD) [56].

2.1.4. PICALM and Autophagy

PICALM plays a key role in multiple steps in autophagy (Figure 2B). *PICALM* regulates the endocytosis of VAMP2, VAMP3 and VAMP8, which are essential for the autophagy process [54]. *PICALM* knockdown leads to a reduced

fusion of autophagosome precursor vesicles, which is a VAMP2- and VAMP3-dependent process, and thus decreases autophagosome biogenesis. PICALM knockdown also influences the process of autophagosome-lysosome fusion, which is a VAMP8-dependent process [57]. Therefore, PICALM reduction results in a general reduction in autophagic flux in HeLa, HEK, CAD and MEF cells [45]. Additionally, PICALM is also implicated in the maturation of Cathepsin D, which is a critical lysosomal component [42]. Disruption of exon1 of *PICALM* leads to deficits in the maturation of Cathepsin D and autophagy in HeLa cells [42].

2.2. Protein Structure of PICALM

PICALM is constituted of an N-terminal ANTH-domain binding phosphatidyl inositol 4,5 bisphosphate (PtdIns(4,5)P₂) and a C-terminal clathrin adaptor domain (Figure 3A) [36][58].

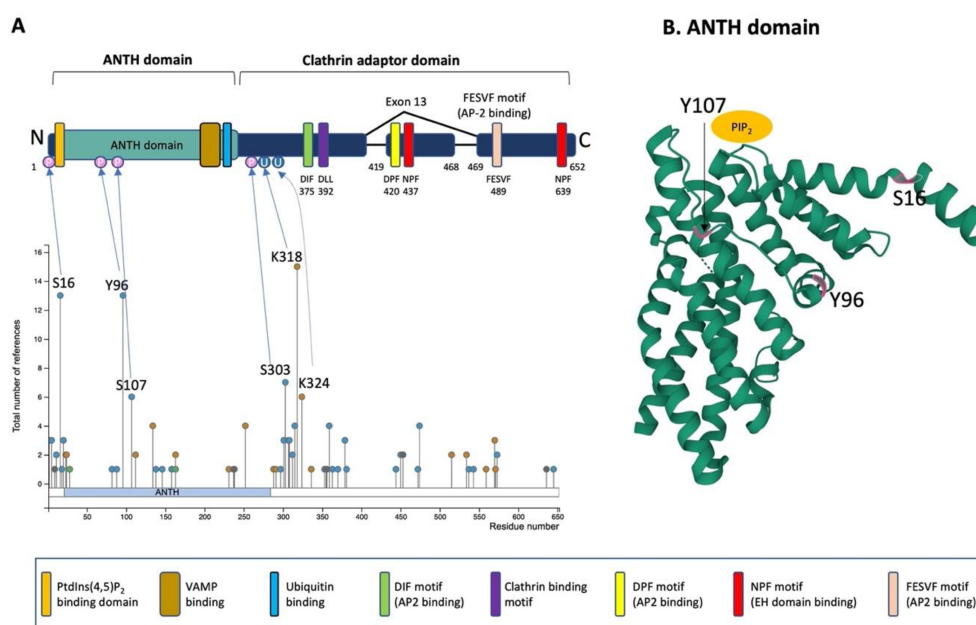


Figure 3. Schematic illustration of PICALM structure and post-translational modifications. **(A)** Schematic structure of PICALM. PICALM is constituted of two distinct domains: the ANTH (AP180 N-terminal homology) domain and clathrin adaptor domain. ANTH domain contains a PtdIns(4,5)P₂ binding domain and vesicle-associated membrane protein (VAMP) binding domain. Clathrin adaptor domain contains several motifs that are critical to interact with endocytic protein, including DIF, DLL Clathrin binding, DPF, NPF and FESVF motifs. DIF motif binds to AP2. DLL motif is conserved and has weak affinity to Clathrin DPF (Aspartic acid-Proline-Phenylalanine) motifs. NPF (Asparagine-Proline-Phenylalanine) motif binds to AP2 and EH domains (Eps15 Homology domain). Four phosphorylation sites (S16, Y96, S107 and S303) and two ubiquitination sites (K318 and K324) have been described in more than 5 references and validated by mass-spectrometry and thus are highlighted [59]. Altogether, the reported PTMs of PICALM are 41 phosphorylation (S2, S5, T11, S16, T18, S20, S23, T82, Y88, Y96, S107, Y138, T146, T158, Y237, S297, T301, S303, S307, S308, T312, S315, S353, T355, T356, S359, T363, T370, T379, S381, S444, S450, S453, T472, S474, K515, K534, S537, S543, T573 and S645), 15 ubiquitination (K24, K112, K134, K163, K231, K252, K288, K291, K318, K324, K336, K515, K559, K570 and K571), 1 acetylation (K28), 1 mono-methylation (R9), 1 di-methylation (R636) and 1 sumoylation (K238). **(B)** Computational modeling of

the structure of PICALM N-terminal ANTH domain is shown (<https://www.rcsb.org/structure/3ZYM> (accessed on 1 June 2022)) [60]. PICALM ANTH domain is constituted of 11 α -helices. Three known phosphorylation sites (S16, Y96 and S107) in ANTH domain are highlighted in pink.

The ANTH domain is a membrane-binding domain with specificity for phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) and acts as a universal adaptor for nucleation of clathrin coats. In silico modeling of N-terminal ANTH domain suggests a structure consisting of 11 α -helices [54] (**Figure 3B**). The PtdIns(4,5)P₂ binding residues (Kx9Kx(K/R)(H/Y) are present on several helices. The PtdIns(4,5)P₂ binding sites contain evolutionally conserved basic amino acid residues (K28, K38, K40 and K41, numbering according to the PICALM isoform of 652 amino acids) forming a positively charged patch on the surface to interact with phosphates of PtdIns(4,5)P₂ [58]. The ANTH domain also contains the binding region for R-SNARE proteins, and PICALM can bind simultaneously PtdIns(4,5)P₂ and VAMP8 [54]. PICALM interacts with R-SNARE proteins via its hydrophobic amino acid residues such as L219, F240, M244, I272 and L251 [54]. A recent study has shown that the ANTH domain directly binds to ubiquitin, and the ANTH domain can act as an adaptor for ubiquitinated protein [61].

In the C-terminal clathrin adaptor domain of PICALM contains critical motifs that allow PICALM to interact with clathrin and other endocytic machinery proteins. The clathrin binding motif is DLLDLQ (392–397 in the isoform of 652 amino acids). AP2-binding motifs are DIF (D375, I376 and F377), DPF (D420, P421 and F422) and FESVF (F489, E490, S491, V492 and F493). The asparagine-proline-phenylalanine (NPF) motif binds to Eps15 homology (EH) domains found in proteins associated with endocytosis and vesicle trafficking. Since exon 13 contains one DPF (AP2 binding) and one NPF (EH domain binding) motif, the splice variant without exon 13 is supposed to have reduced interaction with AP2 and other protein partners owning EH-domains [37].

2.3. Post-Translational Modifications of PICALM

PICALM undergoes several post-translational modifications (PTM), such as phosphorylation, methylation, ubiquitination, sumoylation and acetylation [59][60][62][63][64][65][66][67][68]. These post-translational modifications have been identified on numerous amino acid residues of PICALM: 41 phosphorylation, 15 ubiquitination, 1 acetylation, 1 mono-methylation, 1 di-methylation and 1 sumoylation sites have been described so far. Certain factors are known to induce some of the post-translational modifications on PICALM, such as phosphorylation by A β treatment in primary neuronal culture [69], by BI2536, a selective inhibitor of protein kinase Polo-Like Kinase 1 (PLK1), inducing phosphorylation (Y138, T146 and S537) [70], by ischemia (S303 and S315) [71] and by nocodazole, an inhibitor of microtubule polymerization (S474 and S645) [72]. The most well-characterized PTM of PICALM are the four phosphorylation sites (S16, Y96, S107 and S303) and two ubiquitination sites (K318 and K324) that have been validated by mass-spectrometry and have been described for more than 5 times [59]. While the phosphorylation on the ANTH domain may influence the interactions of PICALM with PtdIns(4,5)P₂ or R-SNARE proteins, the other phosphorylation residues are located in the C-terminal clathrin adaptor domain exhibiting pivotal functions in protein–protein interaction of PICALM with clathrin and other endocytic machinery proteins. Therefore, it is speculated that phosphorylation of PICALM should play a role in regulating interaction with PtdIns(4,5)P₂ and other protein binding partners. For instance, the R-SNARE binding domain contains several sites that can undergo PTM,

such as one phosphorylation (Y237), two ubiquitination (K231 and K252) and one sumoylation (K238), and thus, these PTM may regulate the interaction between PICALM and R-SNARE.

PICALM is a substrate of calpain and caspase [27][73][74]. Nevertheless, it remains largely unknown if the phosphorylation and ubiquitination of PICALM affect its protein stability or degradation.

Given that PICALM contains more than 130 putative phosphorylation sites, PICALM phosphorylation may be dysregulated in AD brains not only in terms of its global phosphorylation level but also in the pattern of site-specific phosphorylation. Indeed, A β exposure increases PICALM phosphorylation in primary rat neuronal cortical culture [69] though phosphorylation sites and the involved kinases remain to be identified. While it is speculated that phosphorylation may change the interaction of PICALM with its binding partners such as R-SNAREs, PtdIns(4,5)P₂ or clathrin, it remains elusive which kinases are involved in PICALM phosphorylation, whether PICALM phosphorylation is misregulated during the progression of AD, whether PICALM phosphorylation has any impact on its protein stability/degradation or its subcellular localization and whether phosphorylation of PICALM may cause a loss of function and/or a gain of toxicity.

References

1. Wong, C.W.; Quaranta, V.; Glenner, G.G. Neuritic Plaques and Cerebrovascular Amyloid in Alzheimer Disease Are Antigenically Related. *Proc. Natl. Acad. Sci. USA* 1985, 82, 8729–8732.
2. Brion, J.P.; Couck, A.M.; Passareiro, E.; Flament-Durand, J. Neurofibrillary Tangles of Alzheimer's Disease: An Immunohistochemical Study. *J. Submicrosc. Cytol.* 1985, 17, 89–96.
3. Voytyuk, I.; De Strooper, B.; Chavez-Gutierrez, L. Modulation of Gamma- and Beta-Secretases as Early Prevention against Alzheimer's Disease. *Biol. Psychiatry* 2018, 83, 320–327.
4. Kamenetz, F.; Tomita, T.; Hsieh, H.; Seabrook, G.; Borchelt, D.; Iwatsubo, T.; Sisodia, S.; Malinow, R. App Processing and Synaptic Function. *Neuron* 2003, 37, 925–937.
5. Long, J.M.; Holtzman, D.M. Holtzman. Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell* 2019, 179, 312–339.
6. Wang, Y.; Mandelkow, E. Tau in Physiology and Pathology. *Nat. Rev. Neurosci.* 2016, 17, 22–35.
7. Goedert, M. Tau Filaments in Neurodegenerative Diseases. *FEBS Lett.* 2018, 592, 2383–2391.
8. Nelson, P.T.; Alafuzoff, I.; Bigio, E.H.; Bouras, C.; Braak, H.; Cairns, N.J.; Castellani, R.J.; Crain, B.J.; Davies, P.; del Tredici, K.; et al. Correlation of Alzheimer Disease Neuropathologic Changes with Cognitive Status: A Review of the Literature. *J. Neuropathol. Exp. Neurol.* 2012, 71, 362–381.
9. Braak, H.; Braak, E. Neuropathological Staging of Alzheimer-Related Changes. *Acta Neuropathol.* 1991, 82, 239–259.

10. Clavaguera, F.; Hench, J.; Goedert, M.; Tolnay, M. Prion-Like Transmission and Spreading of Tau Pathology. *Neuropathol. Appl. Neurobiol.* 2014, 41, 47–58.
11. Buee, L.; Bussiere, T.; Buee-Scherrer, V.; Delacourte, A.; Hof, P.R. Tau Protein Isoforms, Phosphorylation and Role in Neurodegenerative Disorders. *Brain Res. Brain Res. Rev.* 2000, 33, 95–130.
12. Tanzi, R.E. The Genetics of Alzheimer Disease. *Cold Spring Harb. Perspect. Med.* 2012, 2, a006296.
13. Guerreiro, R.; Bras, J. The Age Factor in Alzheimer's Disease. *Genome Med.* 2015, 7, 106.
14. Harold, D.; Abraham, R.; Hollingworth, P.; Sims, R.; Gerrish, A.; Hamshere, M.L.; Pahwa, J.S.; Moskva, V.; Dowzell, K.; Williams, A.; et al. Genome-Wide Association Study Identifies Variants at *Clu* and *Picalm* Associated with Alzheimer's Disease. *Nat. Genet.* 2009, 41, 1088–1093.
15. Lambert, J.C.; Heath, S.; Even, G.; Campion, D.; Sleegers, K.; Hiltunen, M.; Combarros, O.; Zelenika, D.; Bullido, M.J.; Tavernier, B.; et al. Genome-Wide Association Study Identifies Variants at *Clu* and *Cr1* Associated with Alzheimer's Disease. *Nat. Genet.* 2009, 41, 1094–1099.
16. Bellenguez, C.; Kucukali, F.; Jansen, I.E.; Kleindam, L.; Moreno-Grau, S.; Amin, N.; Naj, A.C.; Campos-Martin, R.; Grenier-Boley, B.; Andrade, V.; et al. New Insights into the Genetic Etiology of Alzheimer's Disease and Related Dementias. *Nat. Genet.* 2022, 54, 412–436.
17. Karch, C.M.; Goate, A.M. Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. *Biol. Psychiatry* 2015, 77, 43–51.
18. Dourlen, P.; Kilinc, D.; Malmanche, N.; Chapuis, J.; Lambert, J.C. The New Genetic Landscape of Alzheimer's Disease: From Amyloid Cascade to Genetically Driven Synaptic Failure Hypothesis? *Acta Neuropathol.* 2019, 138, 221–236.
19. Van Acker, Z.P.; Bretou, M.; Annaert, W. Endo-Lysosomal Dysregulations and Late-Onset Alzheimer's Disease: Impact of Genetic Risk Factors. *Mol. Neurodegener.* 2019, 14, 20.
20. Maurano, M.T.; Humbert, R.; Rynes, E.; Thurman, R.E.; Haugen, E.; Wang, H.; Reynolds, A.P.; Sandstrom, R.; Qu, H.; Brody, J.; et al. Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. *Science* 2012, 337, 1190–1195.
21. Gallagher, M.D.; Chen-Plotkin, A.S. The Post-Gwas Era: From Association to Function. *Am. J. Hum. Genet.* 2018, 102, 717–730.
22. Ensembl. November 2021. Available online: <http://www.ensembl.org/ENSG00000073921> (accessed on 1 June 2022).
23. Schnetz-Boutaud, N.C.; Hoffman, J.; Coe, J.E.; Murdock, D.G.; Pericak-Vance, M.A.; Haines, J.L. Identification and Confirmation of an Exonic Splicing Enhancer Variation in Exon 5 of the Alzheimer Disease Associated *Picalm* Gene. *Ann. Hum. Genet.* 2012, 76, 448–453.

24. Parikh, I.; Fardo, D.W.; Estus, S. Genetics of Picalm Expression and Alzheimer's Disease. *PLoS ONE* 2014, 9, e91242.
25. The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000073921-PICALM/tissue> (accessed on 1 June 2022).
26. Baig, S.; Joseph, S.A.; Tayler, H.; Abraham, R.; Owen, M.J.; Williams, J.; Kehoe, P.G.; Love, S. Distribution and Expression of Picalm in Alzheimer Disease. *J. Neuropathol. Exp. Neurol.* 2010, 69, 1071–1077.
27. Ando, K.; Brion, J.P.; Stygelbout, V.; Suain, V.; Authelet, M.; Dedecker, R.; Chanut, A.; Lacor, P.; Lavour, J.; Sazdovitch, V.; et al. Clathrin Adaptor Calm/Picalm Is Associated with Neurofibrillary Tangles and Is Cleaved in Alzheimer's Brains. *Acta Neuropathol.* 2013, 125, 861–878.
28. Zhao, Z.; Sagare, A.P.; Ma, Q.; Halliday, M.R.; Kong, P.; Kisler, K.; Winkler, E.A.; Ramanathan, A.; Kanekiyo, T.; Bu, G.; et al. Central Role for Picalm in Amyloid-Beta Blood-Brain Barrier Transcytosis and Clearance. *Nat. Neurosci.* 2015, 18, 978–987.
29. Xiao, Q.; Gil, S.C.; Yan, P.; Wang, Y.; Han, S.; Gonzales, E.; Perez, R.; Cirrito, J.R.; Lee, J.M. Role of Phosphatidylinositol Clathrin Assembly Lymphoid-Myeloid Leukemia (Picalm) in Intracellular Amyloid Precursor Protein (App) Processing and Amyloid Plaque Pathogenesis. *J. Biol. Chem.* 2012, 287, 21279–21289.
30. Petralia, R.S.; Yao, P.J. Ap180 and Calm in the Developing Hippocampus: Expression at the Nascent Synapse and Localization to Trafficking Organelles. *J. Comp. Neurol.* 2007, 504, 314–327.
31. Chen, W.T.; Lu, A.; Craessaerts, K.; Pavie, B.; Frigerio, C.S.; Corthout, N.; Qian, X.; Lalakova, J.; Kuhnemund, M.; Voytyuk, I.; et al. Spatial Transcriptomics and in Situ Sequencing to Study Alzheimer's Disease. *Cell* 2020, 182, 976–991.e19.
32. Chen, W.T.; Lu, A.; Craessaerts, K.; Pavie, B.; Sala Frigerio, C.; Corthout, N.; Qian, X.; Lalakova, J.; Kuhnemund, M.; Voytyuk, I.; et al. Alzmap. Available online: <https://alzmap.org/> (accessed on 29 April 2018).
33. String. Available online: <https://string-db.org/> (accessed on 1 June 2022).
34. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P.; et al. String V11: Protein-Protein Association Networks with Increased Coverage, Supporting Functional Discovery in Genome-Wide Experimental Datasets. *Nucleic Acids Res.* 2019, 47, D607–D613.
35. Raj, T.; Li, Y.I.; Wong, G.; Humphrey, J.; Wang, M.; Ramdhani, S.; Wang, Y.C.; Ng, B.; Gupta, I.; Haroutunian, V.; et al. Integrative Transcriptome Analyses of the Aging Brain Implicate Altered Splicing in Alzheimer's Disease Susceptibility. *Nat. Genet.* 2018, 50, 1584–1592.

36. Tebar, F.; Bohlander, S.K.; Sorkin, A. Clathrin Assembly Lymphoid Myeloid Leukemia (Calm) Protein: Localization in Endocytic-Coated Pits, Interactions with Clathrin, and the Impact of Overexpression on Clathrin-Mediated Traffic. *Mol. Biol. Cell* 1999, 10, 2687–2702.
37. Meyerholz, A.; Hinrichsen, L.; Groos, S.; Esk, P.C.; Brandes, G.; Ungewickell, E.J. Effect of Clathrin Assembly Lymphoid Myeloid Leukemia Protein Depletion on Clathrin Coat Formation. *Traffic* 2005, 6, 1225–1234.
38. Xu, W.; Tan, L.; Yu, J.T. The Role of Picalm in Alzheimer's Disease. *Mol. Neurobiol.* 2015, 52, 399–413.
39. Miranda, A.M.; Herman, M.; Cheng, R.; Nahmani, E.; Barrett, G.; Micevska, E.; Fontaine, G.; Potier, M.C.; Head, E.; Schmitt, F.A.; et al. Excess Synaptojanin 1 Contributes to Place Cell Dysfunction and Memory Deficits in the Aging Hippocampus in Three Types of Alzheimer's Disease. *Cell Rep.* 2018, 23, 2967–2975.
40. Petralia, R.S.; Wang, Y.X.; Indig, F.E.; Bushlin, I.; Wu, F.; Mattson, M.P.; Yao, P.J. Reduction of Ap180 and Calm Produces Defects in Synaptic Vesicle Size and Density. *Neuromol. Med.* 2013, 15, 49–60.
41. Miller, S.E.; Mathiasen, S.; Bright, N.A.; Pierre, F.; Kelly, B.T.; Kladt, N.; Schauss, A.; Merrifield, C.J.; Stamou, D.; Honing, S.; et al. Calm Regulates Clathrin-Coated Vesicle Size and Maturation by Directly Sensing and Driving Membrane Curvature. *Dev. Cell* 2015, 33, 163–175.
42. Hattersley, K.J.; Carosi, J.M.; Hein, L.K.; Bensalem, J.; Sargeant, T.J. Picalm Regulates Cathepsin D Processing and Lysosomal Function. *Biochem. Biophys. Res. Commun.* 2021, 570, 103–109.
43. Nonet, M.L.; Holgado, A.M.; Brewer, F.; Serpe, C.J.; Norbeck, B.A.; Holleran, J.; Wei, L.; Hartweg, E.; Jorgensen, E.M.; Alfonso, A. Unc-11, a Caenorhabditis Elegans Ap180 Homologue, Regulates the Size and Protein Composition of Synaptic Vesicles. *Mol. Biol. Cell* 1999, 10, 2343–2360.
44. Ishikawa, Y.; Maeda, M.; Pasham, M.; Aguet, F.; Tacheva-Grigorova, S.K.; Masuda, T.; Yi, H.; Lee, S.U.; Xu, J.; Teruya-Feldstein, J.; et al. Role of the Clathrin Adaptor Picalm in Normal Hematopoiesis and Polycythemia Vera Pathophysiology. *Haematologica* 2015, 100, 439–451.
45. Moreau, K.; Fleming, A.; Imarisio, S.; Ramirez, A.L.; Mercer, J.L.; Jimenez-Sanchez, M.; Bento, C.F.; Puri, C.; Zavodszky, E.; Siddiqi, F.; et al. Picalm Modulates Autophagy Activity and Tau Accumulation. *Nat. Commun.* 2014, 5, 4998.
46. Dreyling, M.H.; Martinez-Climent, J.A.; Zheng, M.; Mao, J.; Rowley, J.D.; Bohlander, S.K. The T(10;11)(P13;Q14) in the U937 Cell Line Results in the Fusion of the Af10 Gene and Calm, Encoding a New Member of the Ap-3 Clathrin Assembly Protein Family. *Proc. Natl. Acad. Sci. USA* 1996, 93, 4804–4809.

47. Klebig, M.L.; Wall, M.D.; Potter, M.D.; Rowe, E.L.; Carpenter, D.A.; Rinchik, E.M. Mutations in the Clathrin-Assembly Gene Picalm Are Responsible for the Hematopoietic and Iron Metabolism Abnormalities in Fit1 Mice. *Proc. Natl. Acad. Sci. USA* 2003, 100, 8360–8365.
48. Suzuki, M.; Tanaka, H.; Tanimura, A.; Tanabe, K.; Oe, N.; Rai, S.; Kon, S.; Fukumoto, M.; Takei, K.; Abe, T.; et al. The Clathrin Assembly Protein Picalm Is Required for Erythroid Maturation and Transferrin Internalization in Mice. *PLoS ONE* 2012, 7, e31854.
49. Huang, F.; Khvorova, A.; Marshall, W.; Sorkin, A. Analysis of Clathrin-Mediated Endocytosis of Epidermal Growth Factor Receptor by Rna Interference. *J. Biol. Chem.* 2004, 279, 16657–16661.
50. Harel, A.; Wu, F.; Mattson, M.P.; Morris, C.M.; Yao, P.J. Evidence for Calm in Directing Vamp2 Trafficking. *Traffic* 2008, 9, 417–429.
51. Mercer, J.L.; Argus, J.P.; Crabtree, D.M.; Keenan, M.M.; Wilks, M.Q.; Chi, J.T.; Bensinger, S.J.; Lavau, C.P.; Wechsler, D.S. Modulation of Picalm Levels Perturbs Cellular Cholesterol Homeostasis. *PLoS ONE* 2015, 10, e0129776.
52. Bushlin, I.; Petralia, R.S.; Wu, F.; Harel, A.; Mughal, M.R.; Mattson, M.P.; Yao, P.J. Clathrin Assembly Protein Ap180 and Calm Differentially Control Axogenesis and Dendrite Outgrowth in Embryonic Hippocampal Neurons. *J. Neurosci.* 2008, 28, 10257–10271.
53. Koo, S.J.; Markovic, S.; Puchkov, D.; Mahrenholz, C.C.; Beceren-Braun, F.; Maritzen, T.; Dervedde, J.; Volkmer, R.; Oschkinat, H.; Haucke, V. Snare Motif-Mediated Sorting of Synaptobrevin by the Endocytic Adaptors Clathrin Assembly Lymphoid Myeloid Leukemia (Calm) and Ap180 at Synapses. *Proc. Natl. Acad. Sci. USA* 2012, 108, 13540–13545.
54. Miller, S.E.; Sahlender, D.A.; Graham, S.C.; Honing, S.; Robinson, M.S.; Peden, A.A.; Owen, D.J. The Molecular Basis for the Endocytosis of Small R-Snares by the Clathrin Adaptor Calm. *Cell* 2011, 147, 1118–1131.
55. Guo, C.; Ma, Y.Y. Calcium Permeable-Ampa Receptors and Excitotoxicity in Neurological Disorders. *Front. Neural Circuits* 2021, 15, 711564.
56. Azarnia Tehran, D.; Kochlamazashvili, G.; Pampaloni, N.P.; Sposini, S.; Shergill, J.K.; Lehmann, M.; Pashkova, N.; Schmidt, C.; Lowe, D.; Napieczynska, H.; et al. Selective Endocytosis of Ca²⁺-Permeable Ampars by the Alzheimer's Disease Risk Factor Calm Bidirectionally Controls Synaptic Plasticity. *Sci. Adv.* 2022, 8, eabl5032.
57. Menzies, F.M.; Fleming, A.; Rubinsztein, D.C. Compromised Autophagy and Neurodegenerative Diseases. *Nat. Rev. Neurosci.* 2015, 16, 345–357.
58. Ford, M.G.; Pearse, B.M.; Higgins, M.K.; Vallis, Y.; Owen, D.J.; Gibson, A.; Hopkins, C.R.; Evans, P.R.; McMahon, H.T. Simultaneous Binding of Ptdins(4,5)P₂ and Clathrin by Ap180 in the Nucleation of Clathrin Lattices on Membranes. *Science* 2001, 291, 1051–1055.

59. Phosphositeplus. Available online: <https://www.phosphosite.org/> (accessed on 1 June 2022).
60. Hornbeck, P.V.; Kornhauser, J.M.; Tkachev, S.; Zhang, B.; Skrzypek, E.; Murray, B.; Latham, V.; Sullivan, M. Phosphositeplus: A Comprehensive Resource for Investigating the Structure and Function of Experimentally Determined Post-Translational Modifications in Man and Mouse. *Nucleic Acids Res.* 2012, 40, D261–D270.
61. Pashkova, N.; Gakhar, L.; Yu, L.; Schnicker, N.J.; Minard, A.Y.; Winistorfer, S.; Johnson, I.E.; Piper, R.C. Anth Domains within Calm, Hip1r, and Sla2 Recognize Ubiquitin Internalization Signals. *eLife* 2021, 10, e72583.
62. Larsen, S.C.; Sylvestersen, K.B.; Mund, A.; Lyon, D.; Mullari, M.; Madsen, M.V.; Daniel, J.A.; Jensen, L.J.; Nielsen, M.L. Proteome-Wide Analysis of Arginine Monomethylation Reveals Widespread Occurrence in Human Cells. *Sci. Signal* 2016, 9, rs9.
63. Mertins, P.; Mani, D.R.; Ruggles, K.V.; Gillette, M.A.; Clauser, K.R.; Wang, P.; Wang, X.; Qiao, J.W.; Cao, S.; Petralia, F.; et al. Proteogenomics Connects Somatic Mutations to Signalling in Breast Cancer. *Nature* 2016, 534, 55–62.
64. Schweppe, D.K.; Rigas, J.R.; Gerber, S.A. Quantitative Phosphoproteomic Profiling of Human Non-Small Cell Lung Cancer Tumors. *J. Proteom.* 2013, 91, 286–296.
65. Sharma, K.; D'Souza, R.C.; Tyanova, S.; Schaab, C.; Wisniewski, J.R.; Cox, J.; Mann, M. Ultradeep Human Phosphoproteome Reveals a Distinct Regulatory Nature of Tyr and Ser/Thr-Based Signaling. *Cell Rep.* 2014, 8, 1583–1594.
66. Shiromizu, T.; Adachi, J.; Watanabe, S.; Murakami, T.; Kuga, T.; Muraoka, S.; Tomonaga, T. Identification of Missing Proteins in the Nextprot Database and Unregistered Phosphopeptides in the Phosphositeplus Database as Part of the Chromosome-Centric Human Proteome Project. *J. Proteome Res.* 2013, 12, 2414–2421.
67. Zhou, H.; di Palma, S.; Preisinger, C.; Peng, M.; Polat, A.N.; Heck, A.J.; Mohammed, S. Toward a Comprehensive Characterization of a Human Cancer Cell Phosphoproteome. *J. Proteome Res.* 2013, 12, 260–271.
68. Mertins, P.; Qiao, J.W.; Patel, J.; Udeshi, N.D.; Clauser, K.R.; Mani, D.R.; Burgess, M.W.; Gillette, M.A.; Jaffe, J.D.; Carr, S.A. Integrated Proteomic Analysis of Post-Translational Modifications by Serial Enrichment. *Nat. Methods* 2013, 10, 634–637.
69. Henriques, A.G.; Muller, T.; Oliveira, J.M.; Cova, M.; da Cruz, E.S.C.B.; da Cruz, E.S.O.A. Altered Protein Phosphorylation as a Resource for Potential Ad Biomarkers. *Sci. Rep.* 2016, 6, 30319.
70. Kettenbach, A.N.; Schweppe, D.K.; Faherty, B.K.; Pechenick, D.; Pletnev, A.A.; Gerber, S.A. Quantitative Phosphoproteomics Identifies Substrates and Functional Modules of Aurora and Polo-Like Kinase Activities in Mitotic Cells. *Sci. Signal* 2011, 4, rs5.

71. Mertins, P.; Yang, F.; Liu, T.; Mani, D.R.; Petyuk, V.A.; Gillette, M.A.; Clauser, K.R.; Qiao, J.W.; Gritsenko, M.A.; Moore, R.J.; et al. Ischemia in Tumors Induces Early and Sustained Phosphorylation Changes in Stress Kinase Pathways but Does Not Affect Global Protein Levels. *Mol. Cell Proteom.* 2014, 13, 1690–1704.
72. Olsen, J.V.; Vermeulen, M.; Santamaria, A.; Kumar, C.; Miller, M.L.; Jensen, L.J.; Gnad, F.; Cox, J.; Jensen, T.S.; Nigg, E.A.; et al. Quantitative Phosphoproteomics Reveals Widespread Full Phosphorylation Site Occupancy During Mitosis. *Sci. Signal* 2010, 3, ra3.
73. Kim, J.A.; Kim, H.L. Cleavage of Purified Neuronal Clathrin Assembly Protein (Calm) by Caspase 3 and Calpain. *Exp. Mol. Med.* 2001, 33, 245–250.
74. Rudinskiy, N.; Grishchuk, Y.; Vaslin, A.; Puyal, J.; Delacourte, A.; Hirling, H.; Clarke, P.G.; Luthi-Carter, R. Calpain Hydrolysis of Alpha- and Beta2-Adaptins Decreases Clathrin-Dependent Endocytosis and May Promote Neurodegeneration. *J. Biol. Chem.* 2009, 284, 12447–12458.

Retrieved from <https://encyclopedia.pub/entry/history/show/87801>