Introduction

Ubiquitin-dependent proteolysis is a major mechanism that downregulates misfolded proteins or those that have finished a programmed task. In the last two decades, neddylation has emerged as a major regulatory pathway for ubiquitination. Central to the neddylation pathway is the amyloid precursor protein (APP)-binding protein APP-BP1, which together with Uba3, plays an analogous role to the ubiquitin-activating enzyme E1 in nedd8 activation. Activated nedd8 covalently modifies and activates a major class of ubiquitin ligases called Cullin-RING ligases (CRLs). New evidence suggests that neddylation also modifies Type-1 transmembrane receptors such as APP. Here we review the functions of neddylation and summarize evidence suggesting that dysfunction of neddylation is involved in Alzheimer’s disease.

Abstract

Ubiquitin-dependent proteolysis is a major mechanism that downregulates misfolded proteins or those that have finished a programmed task. In the last two decades, neddylation has emerged as a major regulatory pathway for ubiquitination. Central to the neddylation pathway is the amyloid precursor protein (APP)-binding protein APP-BP1, which together with Uba3, plays an analogous role to the ubiquitin-activating enzyme E1 in nedd8 activation. Activated nedd8 covalently modifies and activates a major class of ubiquitin ligases called Cullin-RING ligases (CRLs). New evidence suggests that neddylation also modifies Type-1 transmembrane receptors such as APP. Here we review the functions of neddylation and summarize evidence suggesting that dysfunction of neddylation is involved in Alzheimer’s disease.

Keywords: APP • NAE1 • Alzheimer’s disease • Parkinson’s disease • neurodegeneration • proteasome • neurogenesis • ubiquitination • neddylation • differentiation

Neddylation in APP proteolysis and signaling

Dysfunction of neddylation in AD

Neddylation in AD neurogenesis

Conclusions

Introduction

Ubiquitination tags a target protein for proteolysis when it is misfolded or when it finishes a programmed task. It also plays a role in cell signaling depending on the ubiquitin-linkage type. Ubiquitination is important in maintaining normal cell functions in all types of cells, most relevantly those in the brain. Evidence accumulated in recent years suggests that ubiquitination dysfunction underlies major neurodegenerative diseases. This evidence not only includes the presence of insoluble proteins such as tau tangles and amyloid plaques in Alzheimer’s disease (AD), but also the critical roles certain proteins play in the ubiquitination pathway. One such important pathway protein is APP-binding protein 1 APP-BP1 (BP1), which activates CRLs and regulates Type-1 transmembrane receptor signaling via neddylation. Neddylation has been under intense scrutiny in cancer research (see recent review by Soucy et al. [1]). This current review instead focuses on the emerging field that studies the neuronal functions of APP-BP1 (BP1) and its downstream effectors. We highlight the areas of research that suggests that the BP1-activated neddylation pathway is dysfunctional in AD.

BP1 was first cloned by its interaction with the C-terminus of APP in 1996. It is homologous to Arabidopsis auxin-resistant gene 1 (AXR1) [2, 3]. The ubiquitin-like protein nedd8 was first cloned as a developmentally down-regulated gene expressed in neural precursor cells in 1993 [4]. Both BP1 and AXR1 are homologous to the N-terminus of the ubiquitin-activating enzyme E1 except that they lack the active site cysteine residue required for ubiquitin conjugation and present in the C-terminus of E1. In 1998, Ula1, the yeast counterpart
of BP1 and AXR1, was shown to form a heterodimer with Uba1 (analogous to mammalian Uba3) to activate Rub1 (related to ubiquitin-1, analogous to ned8) [5]. Based on in vitro studies, BP1 was proposed to be a member of the ned8-conjugation pathway [6, 7]. In vitro, BP1 together with Uba3 that has an active cysteine and the ATP binding site, behaves like the ubiquitin-activating enzyme E1 and activates ned8 (Fig. 1). After activation, ned8 forms a thiolester bond with the ned8-conjugating enzyme Ubc12. Subsequently, ned8 is covalently coupled to the substrate in a step mediated by a ned8 ligase such as the RING domain protein Rbx1 [8, 9], Rbx2 [10], and Tfs3 [11]; an IAP (inhibitor of apoptosis protein) [12, 13]; and Dcn1 (the defective in Cullin neddylation 1) or Dcn1-like protein [14, 15]. The BP1/Uba3 bipartite activating enzyme of ned8 is also known as NAE (the ned8-activating enzyme). The series of reactions that lead to the covalent conjugation of ned8 to the substrate is now known as neddylation.

The function of BP1 in cell growth was first characterized in ts41 cell line, a mutant of V-79 cell line. These cells undergo successive DNA replication without cell division at the non-permissive temperature [16]. In 1992, temperature-shift experiments by Handel and Weintraub confirmed that the ts41 gene product promotes entry into mitosis but also discovered that it inhibits entry into S phase [17]. An informal communication in 1997 suggests that the ts41 gene encodes the hamster BP1 gene [18]. In 2000, we showed that human BP1 rescues the ts41 gene defect at the S-M checkpoint in a manner dependent on the neddylation pathway [19]. This study provided the first evidence that inhibition of BP1 causes cell death, suggesting that it is a promising target for cancer treatment. A small molecule inhibitor of NAE called MLN4924 was subsequently developed for cancer growth inhibition [20]. Like in ts41 cells at the non-permissive temperature, MLN4924 treatment causes apoptotic death in human tumour cells by deregulating S-phase of the cell cycle [1].

Overview of the ubiquitination-proteasomal pathway

Ubiquitin is a conserved 76-amino acid protein that covalently binds to lysine residues in target proteins and to itself via the ubiquitination pathway [21, 22]. The ubiquitination pathway is carried out through the actions of three enzymes: E1 (the ubiquitin-activating enzyme), E2 (the ubiquitin-conjugating enzyme), and E3 (the ubiquitin ligase) (Fig. 1). The E1 hydrolyzes ATP to form a thiolester bond with the C-terminal glycine of ubiquitin and transfers the activated ubiquitin to a cysteine residue in E2. The E2-ubiquitin can either bind to E3 to directly transfer ubiquitin to a substrate protein, or transfer the ubiquitin to E3 to form an E3-ubiquitin intermediate and then transfer the ubiquitin to the substrate. When a protein is ubiquitinated, the C-terminus of ubiquitin becomes conjugated to a lysine on the substrate protein by an isopeptide bond. Ubiquitination can repeat several times to attach additional ubiquitin molecules to the lysine residue of a former ubiquitin, which introduces an ubiquitin chain onto the substrate protein [23]. Polyubiquitin chains linked by Lys-48 are essential for 26S proteasome recognition, which leads to degradation of the substrate by the proteasome [24]. Polyubiquitin chains linked by Lys-6 inhibit ubiquitin-dependent proteinolysis [25]. Membrane receptor monoubiquitination tags the receptor for endocytosis and degradation in the lysosomes [26]. Monoubiquitination of histones is usually associated with signaling and structural marking [27]. Lys-6-linked polyubiquitin chains are involved in signaling [28] and also mark a receptor for endocytosis and lysosomal degradation [29, 30]. The polyubiquitin chain can be disassembled by the activity of deubiquitinating enzymes and the released ubiquitin is reused in the next ubiquitination cycle [31]. The 26S proteasome is responsible for a large fraction of the regulated protein degradation in eukaryotic cells.

Activation of CRLs by neddylation

E3s control the specificity of proteolysis by recognizing specific substrates through protein-protein interactions. They comprise a diverse family of proteins or protein complexes that can be distinguished based on the type of the interaction domain (RING domain, U-box domain, or HECT domain) used to bind E2 ubiquitin-conjugating enzymes and whether they act as a single subunit or multisubunit complex [32]. A major class of RING domain E3s is the multisubunit ubiquitin-ligase CRLs, which consist of a scaffolding Cullin protein, a zinc-chelating RING protein Rbx1 or Rbx2, and a substrate-recognition subunit. In CRLs, the N-terminus of Cullins interacts with Cullin-specific substrate-recognition subunits, usually via adaptor proteins [33, 34]. Seven Cullins (1, 2, 3, 4A, 4B, 5, and 7) in mammalian cells form about 200 unique CRLs. CRLs play essential roles in cell signaling as indicated by their enrichment in protein networks [35] and hundreds of substrates [36]. Figure 1 shows a diagram of the well-characterized Cullin1 (Cul1) CRL (also known as SCF complex) consisting of Cul1, the RING protein Rbx1, the adaptor Skp1, and the F-box (Fbx) protein βTrCP1. This SCF complex recognizes phosphorylated β-catenin for ubiquitination after it binds to βTrCP1 [33].
The central role of BP1 is to initiate the cascade of reactions that neddylates Cullins and activates CRLs. Nedd8 is an 81 amino acid protein 60% identical and 80% homologous to ubiquitin [4]. Immature neddf is processed to its mature, 76 amino acid form by DEN1, which removes 5 amino acids from its C-terminal tail [37]. Nedd8 is activated by the heterodimer BP1/Uba3 [5–7]. Activated neddf covalently binds to a conserved lysine residue located in the C-terminal Cullin domain in Cullin proteins. Neddylation of Cullins activates CRLs by inducing a conformational change in the C-terminus of the Cullin that promotes ubiquitin transfer to the substrate for ubiquitination [38–40]. Cullin neddylation depends on the assemblies of the CRL complex including the RING protein [41] and the substrate [42–45]. CRLs are often assembled and poised for activation by neddylation upon signaling [35].

The best characterized CRL is the SCF complex, in which Cul1 serves as a scaffold and binds Rbx1 to its C-terminus and Skp1 to its N-terminus [33] (Fig. 1). Skp1 in turn interacts with the F-box (Fbx) motif at the N-terminus of the Fbx protein in the SCF complex [46]. The C-termini of Fbx proteins contain protein-protein interaction domains including WD-40, KELCH and leucine-rich repeat domains [47]. These domains can recruit specific substrates to the SCF complex. In 293T cells, about 70% of Cul1 is assembled with Skp1 and presumably Fbx proteins, independent of the neddylation status [35]. In mammals, about 70 genes encode Fbx proteins [47, 48]. It is well-established that SCF downregulates the signaling molecule β-catenin. Deletion of Uba3 [49] or PS1 [50] in mice both result in equal distance from Y682. Among the five lysine residues, K649, K676, K660, K650, and K688 are conserved in APLP2, whereas only K650 is conserved in APLP1 [76–78]. Besides BP1, many other proteins interactions impaire the function of SCF complexes in plants [66]. In C. elegans, cand-1 mutants have phenotypes associated with the loss of the SCF complex but lack phenotypes associated with other specific CRLs [67]. Only a small fraction of Cullins are associated with CAND1 [35]. These findings suggest that CAND1 regulates a subset of Cullins and of other currently unknown regulatory mechanisms may exist.

ASPP2 (apoptosis-stimulating and p53-binding protein-2) is another CRL regulator [68]. ASPP2, also known as 53BP2 or Bbp, is encoded by TP53BP2 and first cloned as a p53-binding protein [69]. The C-terminus of ASPP2 consists of a proline-rich domain, two ankyrin repeats, and an SH3 domain. Bcl2 and p53 bind ASPP2 competitively to the region that contains the ankyrin repeats and the SH3 domain [70]. ASPP2 interacts with BP1 at two different sites, one near the N-terminus within amino acids 332-483, and the second within the C-terminal amino acids 693–1128 [68]. In HeLa cells, ASPP2 decreases the levels of Cul1 whereas the N-terminal fragment of ASPP2 stabilizes Cul1 [68], which suggests that ASPP2 downregulates Cul1 through the N-terminal domain and that the N-terminal fragment of ASPP2 has a dominant-negative effect. A previous study shows that ASPP2 expression impedes cell cycle progression at G2/M in HEK293 cells [70]. Since ASPP2 interacts with and opposes the effect of BP1 in the cycle progression [17, 19], inhibition of neddylation by ASPP2 is probably the underlying mechanism of ASPP2-mediated cell cycle inhibition and tumour suppression, which was recently reviewed in [71]. We have found that the N-terminal fragments of ASPP2 protect neurons from apoptosis by inhibiting DNA synthesis induced by BP1 overexpression [68]. However, the physiological function of ASPP2 in neurons remains to be determined.

Neddylation in APP proteolysis and signaling

Besides Cullins, nedd8 modifies multiple lysine residues in APP and the EGFR receptor (EGFR) in the cytoplasmic domain [72, 73]. The function of APP is under intense investigation due to its involvement in AD (for recent reviews, see Zheng and Koo [74] and Zhang et al. [75]). EGFR stimulates EGFR neddylation, which enhances subsequent ubiquitination and sorting of the receptor for degradation [72]. Like EGFR, neddylation appears to regulate APP endocytosis and degradation although it is not clear if ubiquitination is also involved.

APP is neddylated in all the lysine residues in the APP intracellular domain (AICD) [73] (Fig. 2A). Neddylation of APP is most likely due to the interaction with BP1 since the lysine residues are within the BP1-binding sites in APP [49, 76, 77]. The positioning of these lysine residues suggests that they play important roles in APP function and processing. Three neddylated lysines are found near the γ-secretase cleavage site. Interestingly, the other two lysines are located at an equal distance from Y682. Among the five lysine residues, K649, K676, and K660 are conserved in APLP2, whereas only K650 is conserved in APLP1 (Fig. 2B). In primary neurons, acute BP1 expression downregulates APP, has no significant effect on APLP1, and shows a potential effect in stabilizing APLP2 [76]. Besides BP1, many other proteins
have been found to bind to AICD of APP, but it is not known if these other binding proteins play a role in APP neddylation. For reviews of the other binding partners of APP in AICD, refer to King and Turner [78] and Kerr and Small [79].

APP is sorted to the lysosome for degradation after internalization [80]. We have shown that exogenous expression of a familial AD mutant of APP in neurons causes apoptosis and enlargement of early endosomes, and increases receptor-mediated endocytosis via a pathway dependent on the binding of BP1 to APP [80]. Downregulation of BP1 that inhibits neddylation, results in the accumulation of APP, the β-secretase-cleaved C-terminal fragment of APP, and Aβ in primary neurons [76]. These data suggest that neddylation of APP signals for APP degradation via endocytosis.

Receptor endocytosis attenuates receptor signaling. It has been shown that neddylation of AICD inhibits APP signaling by reducing the interaction between APP and FE65 [73]. The tyrosine residue Y687 in the NPTY motif regulates rapid APP turnover although it does not appear to be an internalization signal [81]. A recent study shows that Y682 is indispensable for the essential function of APP in developmental regulations [82]. Perhaps neddylation of APP involves a conformational change in this region that affects the tyrosine phosphorylation, stability, and signaling of APP.

**Dysfunction of neddylation in AD**

Neddylation is a delicate biological balance of the cell as demonstrated by the effects of CSN and CAND1. Decreased or increased levels of these regulatory molecules both impair CRL activities. It is expected that BP1 manipulations would display similar complexities. In primary neurons, overexpression of BP1 tips the balance to a direction that induces apoptosis, which involves cell cycle reactivation in neurons [19, 49]. Endogenous BP1 protein and mRNA expressions are abundant in some hippocampal pyramidal cells and dentate granule cells in the brain [77] (Fig. 3A). This suggests that BP1 has a physiological function in these cells, which may be mediated by Cullins and membrane receptors including APP and EGFR as mentioned above. SCCRO (squamous cell carcinoma-related oncogene) also known as DCUN1D1, is a Dcn1-like protein and functions as a ligase for nedd8 in the neddylation pathway [83]. In a recent association study, the GG genotype of DCUN1D1 single nucleotide polymorphism (SNP) was found to be a risk factor for the development of frontotemporal lobar degeneration, carrying a fourfold increased risk association [84]. This along with our previous studies suggests that the BP1 pathway plays a pivotal role in the nervous system.

Neddylation has been found to be deregulated in AD. In human brain tissues, BP1 protein levels are elevated in the Triton-insoluble and SDS-soluble fraction in AD tissues compared to that in age-matched controls [19, 49]. In addition, AD conditions change nedd8 localization. In normal hippocampal pyramidal cells and granule cells, nedd8 is located in the nucleus, but in AD hippocampal neurons, most nedd8 immunostaining is present in the cytoplasm (Fig. 3B) [49]. Anti-nedd8 stains neurofibrillary tangles and senile plaques from patients with AD [85, 86]. Moreover, it has been shown that the tau in the tangles is polyubiquitinated [87]. Evidence in the literature also shows that nedd8 can be erroneously incorporated into ubiquitin chains by the ubiquitination enzymes, which may occur under stress conditions and when the nedd8/ubiquitin ratio is increased [88, 89]. It is conceivable that erroneous nedd8 incorporation into tau ubiquitination may also occur due to elevated cytoplasmic nedd8 in AD neurons. Currently, we are investigating if increased nedd8/ubiquitin ratio will lead to impaired function of the targeted proteins such as tau. A potential pathogenic mechanism involving faulty neddylation may underline the major pathological features of AD.

APP undergoes γ-secretase cleavage to generate Aβ. Presenilin is the enzymatic component of the γ-secretase complex that makes the second cut of APP in the production of Aβ [90]. We have shown that APP, BP1, and PS1 co-migrate to a Triton-insoluble, low density, lipid-raft fraction in brain protein extracts [49, 76]. This fraction of lipid rafts mediates Aβ42 genesis [91]. Lipid rafts consist of the fully assembled γ-secretase complex [92]. Besides interacting with APP, BP1 also interacts with Presenilin [76]. This tripartite interaction not only affects signaling as shown by β-catenin regulation [49-51], but also regulates APP processing [76, 90]. BP1 knockdown stabilizes PS1-CTF [76], which appears to be caused by the reduced activity of a SCF complex since BP1 activates SCF by modifying Cul1 with nedd8. In C. elegans, the Presenilin homologue Sel-12 is downregulated by the Fbx protein Sel-10, a subunit of the SCF complex [93].
Sel-10 interacts with PS1 and enhances PS1 ubiquitination, thus decreasing cellular levels of γ-secretase. Co-expression of Sel-10 and APP in HEK293 cells alters the metabolism of APP and increases the production of Aβ peptides [94]. Along this line of evidence, it has been demonstrated that BP1 suppression dramatically increases Aβ levels, especially the levels of Aβ42, in neurons [76]. Together, these data suggest that BP1-activated neddylation of Cul1 and activation of SCF is necessary for the proper processing of APP.

Besides the role of BP1 in modulating γ-secretase activity, BP1 may also control Aβ42 degradation by activating a parkin-containing ligase. Mutations in parkin cause autosomal recessive early onset PD characterized by the loss of midbrain dopamine neurons [95]. Parkin is a RING protein in the ubiquitin ligase that includes Cul1, Skp1, and the Fbx/WD repeat protein hSel-10 [96, 97]. This Parkin-containing ligase also polyubiquitinates misfolded proteins through a Lys-63-linked ubiquitin chain [98, 99]. Parkin deletion in mutant tau mice causes extensive amyloidosis [100]. Parkin expression has also been shown to ubiquitinate intracellular Aβ and promote Aβ clearance through proteasomal degradation and activation of autophagy [101–103]. These data suggest that activation of the parkin-containing ligase may be beneficial.

The effect of the interaction between APP and BP1 likely depends on cell and tissue environments and developmental stages. BP1 has been shown to inhibit G1/S transition in ts41 cells [17], which suggests that the BP1 pathway is critical for maintaining homeostasis of neurons in response to signaling. However, this finding in ts41 cells was challenged by a recent study showing that BP1 suppression impedes G1/S transition in foetal neural stem cells [104]. The different role of BP1 in the G1/S checkpoint of the cell cycle has not been replicated by independent laboratories in ts41 cells or neural stem cells. In Drosophila, overexpression of the APP-like protein APPL inhibits neddylation whereas augmenting the BP1 pathway rescues APPL-induced apoptosis [105]. This contrasts with our studies using rodent primary neurons in which APP induces apoptosis in a BP1-dependent pathway [49, 80]. These results may reflect the difference between APPL and APP and/or experimental systems.

**Neddylation in AD neurogenesis**

If the functions of BP1 in cell cycle progression are applicable to neuronal populations, one may expect that the neddylation pathway regulates stem cell renewal. Currently, a BP1 KO animal model is not available, but Uba3 deletion leads to embryonic lethality at the peri-implantation stage [106]. This, together with the fact that BP1 is necessary for cell cycle progression in ts41 cells, suggests that BP1 KO mice may be embryonically lethal and that humans who harbor mutations that jeopardize the function of BP1 in neddylation may not survive beyond the fetus stage. Despite that one cannot observe the effect of BP1 on neurogenesis in vivo and limited information is available from in vitro studies, evidence suggests that the BP1 pathway plays pivotal roles in neurogenesis by interacting with APP and PS1 (Fig. 4).
BP1-activated neddylation attenuates the signaling of EGFR and APP and affects neuronal differentiation. EGFR attenuation inhibits the cell cycle [107], which may be necessary for a neural progenitor cell (NPC) to undergo differentiation. Growth factor withdrawal is a prerequisite for NPC differentiation [108]. In this context, a higher rate of neuronal differentiation is found in NPCs isolated from APP KO mice when grown in culture [109]. Deletion of PS1 that abolishes Aβ40 production during development also increases neuronal differentiation, which depletes NPCs [110]. Similarly, reduction of PS1 in the adult brain increases neuronal differentiation [111]. These data suggest that deletion of APP and PS1 abolishes the replication potential of the NPCs and leads to the default fate of differentiation. Since BP1-activated neddylation downregulates EGFR and APP signaling, neddylation may increase the potential for neuronal differentiation. This appears to be the case since BP1 knockdown is shown to reduce neuronal differentiation [112]. BP1 has also been shown to promote neurogenesis induced by focal transient ischemia [113].

A characteristic of the APP KO mice is reduced body weight in the adult animals [114, 115], which indicates impaired cell cycle progression and reduced NPC self-renewal. Perhaps APP deletion results in the loss of a proliferative signal. The secreted, α-secretase cleavage product of APP, sAPPα, behaves like a growth factor and stimulates NPC proliferation (see recent review [116]). In addition, Aβ peptides also induces a strong proliferative effect in NPCs [117]. Meanwhile low levels of monomeric Aβ40 promotes neuronal differentiation whereas low levels of Aβ42 induces gliagenesis [117]. In contrast to these positive effects of sAPPα and Aβ peptides in NPCs, AICD has been shown to attenuate NPC differentiation [109, 112]. To summarize the data, the α-secretase cleavage pathway generates sAPPα that favors NPC self-renewal (Fig. 4A) whereas the β- and γ-secretase pathway mainly produces Aβ40 that promotes NPC differentiation (Fig. 4B). BP1 downregulates AICD and ensures non-amyloidogenic processing of APP, both of which promote neuronal differentiation. Currently, how neurogenesis is affected in AD brains remains controversial [118–120]. Clearly more research is warranted before we can successfully apply stem cell therapies for treating neurodegeneration.

Conclusions

A major conclusion of this review is that dysfunction of neddylation is involved in neurodegenerative diseases, especially in AD. BP1 resides in the center of a large network of proteins that organize the post-translational proteome by activating neddylation. Major substrates of neddylation consist of CRLs and Type 1 transmembrane receptors. BP1 and CRLs play major roles in APP trafficking and processing. Specifically, by neddylating APP, BP1 regulates APP turnover and signaling in neurons and NPCs.

This review also summarizes the intricate relationship between Presenilin and BP1 in the regulation of APP processing and suggests that NPCs are another major cell type affected by the disease conditions. More research in this area will inform potential stem cell therapies since neurogenesis is likely impaired by AD conditions. The BP1 inhibitor MLN4924 is currently under clinical trials and its potential impact on neuronal survival, neuronal signaling, and NPC proliferation and differentiation should also be examined if such drugs are used for prolonged treatments of cancer.

Overall, this review suggests that a better understanding of the neddylation pathway is needed for discovering invaluable biomarkers and specific drug targets against AD. At the molecular level, the available data suggest that APP regulates ubiquitination, but it remains to be established what exactly APP does in the ubiquitination pathway. Once we know the physiological function of APP, we may have concrete strategies to prevent and treat AD.

Acknowledgements

This work was supported by a grant from the National Institutes of Health, US (NIH/NIA R01 AG034980) to Y. Chen.

Conflict of interest

The authors declare that there are no conflicts of interest.
References

1. Soucy TA, Dick LR, Smith PG, et al. The NEDD8 Conjugation Pathway and Its Relevance in Cancer Biology and Therapy. Genes Cancer. 2010; 1: 708–16.

2. Lincoln C, Britton JH, Estelle M. Growth and development of the axr1 mutants of Arabidopsis. Plant Cell. 1990; 2: 1071–80.

3. Leyser HM, Lincoln CA, Timpte C, et al. Arabidopsis auxin-resistance gene AXR1 encodes a protein related to ubiquitin-activating enzyme E1. Nature. 1993; 364: 161–4.

4. Kumar S, Yoshida Y, Noda M. Cloning of a cDNA which encodes a novel ubiquitin-like protein. Biochem Biophys Res Commun. 1993; 195: 393–9.

5. Liskowskius D, Doenges G, Matuschewski K, et al. A novel protein modification pathway related to the ubiquitin system. EMBO J. 1998; 17: 2208–14.

6. Osaka F, Kawasaki H, Aida N, et al. A new NEDD8-ligating system for cullin-4A. Genes Dev. 1998; 12: 2263–8.

7. Gong L, Yeh ET. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J Biol Chem. 1999; 274: 12036–42.

8. Ohta T, Michel JJ, Schottelius AJ, et al. ROCA1, a homolog of APC11, represents a activating and conjugating enzymes of the NEDD8-ligating system for cullin-4A. Mol Cell Biol. 2000; 20: 8185–90.

9. Cernac A, Lincoln C, Lammer DT, et al. The SAR1 gene of Arabidopsis acts downstream of the AXR1 gene in auxin response. Development. 1997; 124: 1583–91.

10. Chen Y, McPhie DL, Hershko A, Ciechanover A. The amylod protein precursor-processing protein APP-BP1 drives the cell cycle through the S-M checkpoint and causes apoptosis in neurons. J Biol Chem. 2000; 275: 8929–35.

11. Soucy TA, Smith PG, Mihlollen MA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009; 458: 732–6.

12. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998; 67: 425–79.

13. Villeneuve NF, Lau A, Zhang DD. Regulation of the Nrf2-Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases. Antioxid Redox Signal. 2010; 13: 1699–712.

14. Harrenman T, Taschner M, Sigurdsson S, et al. Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. Proc Natl Acad Sci USA. 2009; 106: 20705–10.

15. van Nocker S, Vierstra RD. Multiquitin chains linked through lysine 48 are abundant in vivo and are competent intermediates in the ubiquitin proteolytic pathway. J Biol Chem. 1993; 268: 24766–73.

16. Shang F, Deng G, Liu Q, et al. Lys6-modified ubiquitin inhibits ubiquitin-dependent protein degradation. J Biol Chem. 2005; 280: 20647–56.

17. Acconcia F, Sigismundo S, Polo S. Ubiquitin in trafficking: the network at work. Exp Cell Res. 2009; 315: 1610–8.

18. Hicke L. Protein regulation by monoubiquitin. Nat Rev Mol Cell Biol. 2001; 2: 195–201.

19. Sun L, Chen ZJ. The novel functions of ubiquitination in signaling. Curr Opin Cell Biol. 2004; 16: 119–26.

20. Duncan LM, Piper S, Dodd RB, et al. Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. EMBO J. 2006; 25: 1635–45.

21. Oztürk FE, Vantii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Annu Rev Biochem. 2009; 78: 363–97.

22. Moon J, Parry G, Estelle M. The ubiquitin-proteasome pathway and plant development. Plant Cell. 2004; 16: 3181–95.

23. Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nat Rev Mol Cell Biol. 2005; 6: 9–20.

24. Bosu DR, Kipreos ET. Cullin-RING ubiquitin ligases: global regulation and activation cycles. Cell Div. 2008; 3: 7.

25. Bennett EJ, Rush J, Gygi SP, et al. Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. Cell. 2010; 143: 951–65.

26. Emanuele MJ, Elia AE, Xu Q, et al. Global Identification of Modular Cullin-RING Ligase Substrates. Cell. 2011; 147: 459–74.

27. Gan-Erdene T, Nagamalleswari K, Yin L, et al. Identification and characterization of DENV1, a denguevirus of the ULP family. J Biol Chem. 2003; 278: 28892–900.

28. Osaka F, Sakai M, Katayama S, et al. Covalent modifier NEDD8 is essential for SCF ubiquitin-ligase in fission yeast. EMBO J. 2000; 19: 3475–84.

29. Duda DM, Bong LA, Scott DC, et al. Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. Cell. 2008; 134: 995–1006.

30. Saha A, Deshaies RJ. Multimodal activation of the ubiquitin ligase SCF by Nedd8 conjugation. Mol Cell. 2008; 32: 21–31.

31. Furukawa M, Zhang Y, McCarville J, et al. The CUL1 C-terminal sequence and ROC1 are required for efficient nucleic accumulation, NEDD8 modification, and ubiquitin ligase activity of CUL1. Mol Cell Biol. 2000; 20: 8185–97.

32. Kawakami T, Chiba T, Suzuki T, et al. NEDD8 recruits E2-ubiquitin to SCF E3 ligase. EMBO J. 2001; 20: 4003–12.

33. Read MA, Brownell JE, Gladysheva TB, et al. Nedd8 modification of cul-1 activates SCF(beta(TrCP))-dependent ubiquitination of I kappa B alpha. Mol Cell Biol. 2000; 20: 2326–33.
44. Sufan RI, Ohn M. Role of the NEDD8 modification of Cul2 in the sequential activation of ECV complex. Neoplasia. 2006; 8: 956–63.
45. Chew EH, Hagen T. Substrate-mediated regulation of cullin neddylation. J Biol Chem. 2007; 282: 17032–40.
46. Zheng N, Schulman BA, Song L, et al. Structure of the Cul1-Rbx1-Skp1-F box-Skp2 SCF ubiquitin ligase complex. Nature. 2002; 416: 703–9.
47. Gagne JM, Downes BP, Shiou SH, et al. The F-box subunit of the SCF ε3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci USA. 2002; 99: 11519–24.
48. Jin J, Cardozo T, Lovering RC, et al. Systematic analysis and nomenclature of mammalian F-box proteins. Genes Dev. 2004; 18: 2573–80.
49. Chen Y, Liu W, McPhie DL, et al. APP-BP1 mediates APP-induced apoptosis and DNA synthesis and is increased in Alzheimer’s disease brain. J Cell Biol. 2003; 163: 27–33.
50. Xia X, Qian S, Soriano S, et al. Loss of presenilin 1 is associated with enhanced beta-catenin signaling and skin tumorigenesis. Proc Natl Acad Sci USA. 2001; 98: 10863–8.
51. Chen Y, Bodles AM. Amyloid precursor protein modulates beta-catenin degradation. J Neuroinflammation. 2007; 4: 29.
52. Wang F, Deng XW. Plant ubiquitin-proteasome pathway and its role in gibberellin signaling. Cell Res. 2011; 21: 1286–94.
53. Cope GA, Deshaies RJ. COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. Cell. 2003; 114: 663–71.
54. Wolf DA, Zhou C, Wee S. The COP9 signalosome: an assembly and maintenance platform for cullin ubiquitin ligases? Nat Cell Biol. 2003; 5: 1029–33.
55. Wei N, Serino G, Deng XW. The COP9 signalosome: more than a protease. Trends Biochem Sci. 2008; 33: 592–600.
56. Schwechheimer C. The COP9 signalosome (CSN): an evolutionary conserved proteolytic regulator in eukaryotic development. Biochim Biophys Acta. 2004; 1695: 45–54.
57. Kato JY, Yonedo-Kato N. Mammalian COP9 signalosome. Cells Genes. 2009; 14: 1209–25.
58. Lyapina S, Cope G, Shevchenko A, et al. Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. Science. 2001; 292: 1382–5.
59. Cope GA, Suh GS, Aravind L, et al. Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science. 2002; 298: 608–11.
60. Serino G, Deng XW. The COP9 signalosome: regulating plant development through the control of proteolysis. Annu Rev Plant Biol. 2003; 54: 165–82.
61. Wee S, Geyer RK, Toda T, et al. CSN facilitates Cullin-RING ubiquitin ligase function by counteracting autoubiquitcatalytic adapter instability. Nat Cell Biol. 2005; 7: 387–91.
62. Wu JT, Lin HC, Hu YC, et al. Nedd8ylation and deneddylation regulate Cul1 and Cul2 protein accumulation. Nat Cell Biol. 2005; 7: 1014–20.
63. Zheng J, Yang X, Harrell JM, et al. CAN1 binds to unneddylated Cul1 and regulates the formation of SCF ubiquitin ε3 ligase complex. Mol Cell. 2002; 10: 1519–26.
64. Liu J, Furukawa M, Matsumoto T, et al. NEDD8 modification of CUL1 dissociates p120(CAN1), an inhibitor of CUL1-SKP1 binding and SCF ligases. Mol Cell. 2002; 10: 1511–8.
65. Oshikawa K, Matsumoto M, Yada M, et al. Preferential interaction of Tip120A with Cul1 that is not modified by NEDD8 and does not associated with Skp1. Biochem Biophys Res Commun. 2003; 303: 1209–16.
66. Zhang W, Ito H, Quint M, et al. Genetic analysis of CAN1-CUL1 interactions in Arabidopsis supports a role for CAN1-mediated cycling of the SCFTIR1 complex. Proc Natl Acad Sci USA. 2008; 105: 8470–5.
67. Bosu DR, Feng H, Min K, et al. C elegans CAND-1 regulates cullin neddylation, cell proliferation and morphogenesis in specific tissues. Dev Biol. 2010; 346: 113–26.
68. Chen Y, Liu W, Naumovski L, et al. ASP2 inhibits APP-BP1-mediated NEDD8 conjugation to cullin-1 and decreases APP-BP1-induced cell proliferation and neuronal apoptosis. J Neurochem. 2003; 85: 601–9.
69. Iwabuchi K, Bartel PL, Li B, et al. Two cellular proteins that bind to wild-type but not mutant p35. Proc Natl Acad Sci USA. 1994; 91: 6098–102.
70. Naumovski L, Cleary ML. The p53-binding protein 53BP2 also interacts with Bcl2 and impedes cell cycle progression at G2/M. Mol Cell Biol. 1996; 16: 3844–92.
71. Kampa KM, Bonin M, Lopez CD. New insights into the expanding complexity of the tumor suppressor ASPP2. Cell Cycle. 2009; 8: 2871–6.
72. Oved S, Mossesson Y, Zhang Y, et al. Conjugation to Nedd8 instigates ubiquitylation and down-regulation of activated receptor tyrosine kinases. J Biol Chem. 2006; 281: 21640–51.
73. Lee MR, Lee D, Shin SK, et al. Inhibition of APP intracellular domain (AICD) transcriptional activity via covalent conjugation with Nedd8. Biochem Biophys Res Commun. 2008; 366: 976–81.
74. Zheng H, Koo EH. Biology and pathophysiology of the amyloid precursor protein. Mol Neurodegener. 2011; 6: 27.
75. Zhang H, Ma Q, Zhang YW, et al. Proteolytic processing of Alzheimer’s beta-amyloid precursor protein. J Neurochem. 2011; 1: 9–21.
76. Chen Y, Bodles AM, McPhie DL, et al. APP-BP1 inhibits Aβ42 levels by interacting with Presenilin-1. Mol Neurodegener. 2007; 2: 3.
77. Chow N, Korenberg JR, Chen MX, et al. APP-BP1, a novel protein that binds to the carboxy-terminal region of the amyloid precursor protein. J Biol Chem. 1996; 271: 11339–46.
78. King GD, Scott Turner R. Adaptor protein interactions: modulators of amyloid precursor protein metabolism and Alzheimer’s disease risk? Exp Neurol. 2004; 185: 208–19.
79. Kerr ML, Small DH. Cytoplasmic domain of the beta-amyloid precursor protein of Alzheimer’s disease: function, regulation of proteolysis, and implications for drug development. J Neurosci Res. 2005; 80: 151–9.
80. Lallenfeld D, Patzek LJ, McPhie DL, et al. Rats mediates an amyloid precursor protein signaling pathway that leads to apoptosis. J Neurosci. 2007; 27: 7141–53.
81. Perez RG, Soriano S, Hayes JD, et al. Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Aβ42. J Biol Chem. 1999; 274: 18851–6.
82. Barbagallo AP, Wang Z, Zheng H, et al. A single tyrosine residue in the amyloid precursor protein intracellular domain is essential for developmental function. J Biol Chem. 2011; 286: 8717–21.
83. Kim SY, Bommeije LC, Lee BE, et al. SCCRG (DCUN1D1) is an essential component of the E3 complex for neddylation. J Biol Chem. 2008; 283: 33211–20.
84. Villa CJ, Venturrelli E, Fenoglio C, et al. DCUN1D1 is a risk factor for frontotemporal lobar degeneration. Eur J Neurol. 2009; 16: 870–3.
85. Di Kuazi A, Kito K, Abe Y, et al. NEDD8 protein is involved in ubiquitinated inclusion bodies. J Pathol. 2003; 199: 259–66.
inclusions of neurodegenerative disorders. Neuropathol Appl Neurobiol. 2005; 31: 53–61.
87. Cripps D, Thomas SN, Jeng Y, et al. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. J Biol Chem. 2006; 281: 10825–38.
88. Leidecker O, Matic I, Mahata B, et al. The ubiquitin E1 enzyme Ube1 mediates NEDD8 activation under diverse stress conditions. Cell Cycle. 2012; 11: 1142–50.
89. Hjerpe R, Thomas Y, Kurz T. NEDD8 overexpression should not be used to identify substrates of the NEDD8 pathway. J Mol Biol. 2012; 421: 27–9.
90. Wolfe MS. The gamma-Secretase Complex: Membrane-Embedded Proteolytic Ensemble. Biochemistry. 2006; 45: 7931–9.
91. Erehalt R, Keller P, Haass C, et al. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. J Cell Biol. 2003; 160: 113–23.
92. Vetrivel KS, Cheng H, Lin W, et al. Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. J Biol Chem. 2004; 279: 44945–54.
93. Wu G, Hubbard EJ, Kitajewski JK, et al. Evidence for functional and physical association between Caenorhabditis elegans SEL-10, a Cdc4p-related protein, and SEL-12 presenilin. Proc Natl Acad Sci USA. 1998; 95: 15787–91.
94. Li J, Pauley AM, Myers RL, et al. SEL-10 interacts with presenilin 1, facilitates its ubiquitination, and alters A-beta peptide production. J Neurochem. 2002; 82: 1540–8.
95. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998; 392: 605–8.
96. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet. 2000; 25: 302–5.
97. Staropoli JF, McDermott C, Martinat C, et al. Parkin is a component of an SCF-like ubiquitin ligase complex and protects post-mitotic neurons from kainate excitotoxicity. Neuron. 2003; 37: 735–49.
98. Imai Y, Soda M, Takahashi R, Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. J Biol Chem. 2000; 275: 35661–4.
99. Ollmann JA, Li L, Chudaev MV, et al. Parkin-mediated K63-linked polyubiquitination targets misfolded DJ-1 to aggresomes via binding to HDAC6. J Cell Biol. 2007; 178: 1025–35.
100. Rodriguez-Navarro JA, Gomez A, Rodal I, et al. Parkin reverses intracellular beta-amyloid accumulation and its negative effects on proteasome function. J Neurosci Res. 2010; 88: 167–78.
101. Burns MP, Zhang L, Rebeck GW, et al. Parkin promotes intracellular Abeta1-42 clearance. Hum Mol Genet. 2009; 18: 3206–16.
102. Rosen KM, Moussa CE, Lee HK, et al. Parkin reverses intracellular beta-amyloid accumulation and its negative effects on proteasome function. J Neurosci Res. 2010; 88: 167–78.
103. Khandelwal PJ, Herman AM, Hoe HS, et al. Parkin mediates beclin-dependent autophagic clearance of defective mitochondria and ubiquitinated Abeta in AD models. Hum Mol Genet. 2011; 20: 2091–102.
104. Joo Y, Ha S, Hong BH, et al. Amyloid precursor protein binding protein-1 knockdown reduces neuronal differentiation in fetal neural stem cells. NeuroReport. 2012; 23: 61–6.
105. Joo Y, Lee SH, Ha S, et al. Focal transient ischemia increases APP-BP1 expression in neural progenitor cells. NeuroReport. 2011; 22: 200–5.
106. Zheng H, Jiang M, Trumbauer ME, et al. Beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell. 1995; 81: 525–31.
107. Tremml P, Lipp HP, Muller U, et al. Neurobehavioral development, adult openfield exploration and swimming navigation learning in mice with a modified beta-amyloid precursor protein gene. Behav Brain Res. 1998; 95: 65–76.
108. Chasseigneaux S, Allingou B, Functions of Abeta, SAPPalpha and SAPPbeta: similarities and differences. J Neurochem. 2012; 120 Suppl 1: 99–108.
109. Chen Y, Dong C, Abeta40 promotes neuronal cell fate in neural progenitor cells. Cell Death Differ. 2009; 16: 386–94.
110. Jin K, Peel AL, Mao XD, et al. Increased hippocampal neurogenesis in Alzheimer’s disease. Proc Natl Acad Sci USA. 2004; 101: 343–7.
111. Ziabreva I, Perry E, Perry R, et al. Altered neurogenesis in Alzheimer’s disease. J Psychosom Res. 2006; 61: 311–6.
112. Boekhoff K, Joels M, Lucassen PJ. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol Dis. 2006; 24: 1–14.