

# Ubiquilin-1 and protein quality control in Alzheimer disease

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**S**ingle nucleotide polymorphisms in the Ubiquilin-1 gene may confer risk for late-onset Alzheimer disease (AD). We have shown previously that ubiquilin-1 functions as a molecular chaperone for the amyloid precursor protein (APP) and that protein levels of ubiquilin-1 are decreased in the brains of AD patients. We have recently found that ubiquilin-1 regulates APP trafficking and subsequent secretase processing by stimulating non-degradative ubiquitination of a single lysine residue in the cytosolic domain of APP. Thus, ubiquilin-1 plays a central role in regulating APP biosynthesis, trafficking and ultimately toxicity. As ubiquilin-1 and other ubiquilin family members have now been implicated in the pathogenesis of numerous neurodegenerative diseases, these findings provide mechanistic insights into the central role of ubiquilin proteins in maintaining neuronal proteostasis.

## Introduction

Alzheimer disease (AD) is the most common cause of progressive age-associated dementia. AD is characterized neuropathologically by the presence of amyloid plaques composed of an aggregated peptide termed A $\beta$ . The discovery of A $\beta$  and the gene encoding its precursor, amyloid precursor protein (APP), formed the basis of the amyloid cascade hypothesis, which postulates that A $\beta$  production and aggregation are critical events in the pathogenesis of AD. An extensive body of research offers strong evidence that APP and its proteolytic products are central to AD pathogenesis, particularly in rare, dominantly inherited

forms of the disease. Thus, determining the molecular mechanisms that regulate APP trafficking, degradation, processing and subsequent A $\beta$  generation are critical to understanding the mechanisms of synaptic dysfunction and neuronal loss in AD.

## Genetics of AD and Potential Contribution of Ubiquilin-1

Our current understanding of the pathogenesis of AD is largely based on the study of rare early-onset dominantly inherited forms of the disease, collectively known as familial AD (FAD). FAD is caused by alterations in the proteolytic processing of APP. APP can be cleaved by secretase enzymes generating proteolytic fragments of various lengths. Amyloidogenic processing of APP occurs via successive proteolysis by  $\beta$ -secretase and  $\gamma$ -secretase to produce a large secreted ectodomain fragment, the A $\beta$  peptide and an intracellular domain that is released into the cytosol (AICD, APP intracellular domain). FAD is caused by mutations in the APP molecule or the presenilin proteins, which constitute the catalytic core of  $\gamma$ -secretase, all of which promote the formation of a particularly amyloidogenic version of the A $\beta$  peptide (termed A $\beta_{42}$ ). FAD cases account for less than 10% of all AD cases, and little is known about the causes of sporadic late-onset AD. So far, inheritance of the  $\epsilon 4$  allele of the *APOE* gene is the only established genetic risk factor for developing late-onset AD<sup>1,2</sup> and thus there has been intense interest in identifying additional genetic loci associated with late forms of the disease. A positional candidate gene approach reported that single

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nucleotide polymorphisms in the *UBQLN1* gene have a family-based association with late-onset AD.<sup>3</sup> It was suggested that polymorphisms in the *UBQLN1* gene lead to changes in alternative splicing. However, the exact molecular mechanism by which the protein product of this gene, ubiquilin-1, contributes to disease pathogenesis remains to this day unclear. Furthermore, several other studies have failed to identify a genetic linkage of *UBQLN1* with AD in other patient populations.<sup>4-8</sup> Regardless, we have recently demonstrated that AD was associated with decreased ubiquilin-1 protein levels irrespective of *UBQLN1* genotype,<sup>9</sup> suggesting that a more complex combination of genetic and environmental factors may ultimately lead to alterations in ubiquilin-1 function.

### Ubiquilin-1 Structure

Ubiquilin-1 is a member of a superfamily of proteins containing an N-terminal ubiquitin-like domain (UBL) and a C-terminal ubiquitin-associated domain (UBA). Both domains have been implicated in targeting proteins for degradation by the proteasome.<sup>10,11</sup> The UBL domain directly interacts with the S5a/Rpn10/p54 subunit of the proteasome,<sup>10</sup> while the UBA domain binds mono and polyubiquitinated substrates. The central region of ubiquilin contains Sti1 motifs implicated in protein-protein interactions,<sup>12</sup> and these motifs have been shown to possess molecular chaperone activity.<sup>13</sup> We have recently shown that ubiquilin displays molecular chaperone function toward the model clients citrate synthase and firefly luciferase and, more relevantly, toward APP.<sup>9</sup> Furthermore, we found that the Sti1 domains of ubiquilin-1 bind APP early in the secretory pathway and prevent inappropriate intermolecular interactions of the AICD, demonstrating the ability of ubiquilin-1 to prevent the aggregation of AICD/APP in *in vitro* and in cellular models.<sup>9</sup>

### Protein Ubiquitination and Regulation by UBL/UBA Domain-Containing Proteins

Ubiquitin is a 76 amino acid protein which is covalently attached to the

$\epsilon$ -amino group of lysine residues on target proteins via an isopeptide bond. Ubiquitin can be conjugated as a single molecule. Alternatively, additional ubiquitin molecules can be further conjugated to one of several lysine residues present on the previously attached ubiquitin moiety itself to make a polyubiquitin chain. Polyubiquitin chains conjugated to lysine 29 (K29) or lysine 48 (K48) of ubiquitin constitute a degradative signal, whereas monoubiquitin and chains conjugated to lysine 63 (K63) function in a signaling capacity.<sup>14</sup> These reactions are catalyzed by ubiquitin E2 and E3 ligases, with the latter important in determining substrate specificity. RING finger E3 ligases function as scaffolds to bring E2 enzymes in close proximity to substrates and are representative of the majority of E3s in the human genome.<sup>15</sup> Once a polypeptide chain is modified by a ubiquitin molecule, it has the potential to be bound by a UBA domain-containing protein.

UBA domains can bind to a variety of ubiquitin modifications, including K48/K63 and monoubiquitin.<sup>16</sup> The UBA domains of ubiquilin-1 and its fungal homolog Dsk2 are quite promiscuous and can bind mono, K48 and K63 chains with equivalent affinities.<sup>17,18</sup> The functions of UBA domains are quite diverse, but are best characterized for the DNA repair protein and ubiquilin homolog hRad23. K48 ubiquitination of hRad23 substrates results in hRad23 binding via the UBA domain. The substrate is subsequently recruited to the S5a subunit of the proteasome via the UBL domain of hRad23. Thus, hRad23 is thought to function as a “shuttle” that delivers ubiquitinated substrates to the proteasome.<sup>19</sup> Counterintuitively, hRad23 has also been shown to stabilize substrates and allow accumulation of polyubiquitinated proteins.<sup>20</sup> This finding has now been demonstrated for numerous other UBA domain containing proteins. This mechanism may be related to the ability of UBA domains to “cap” small chain ubiquitinated substrates preventing further chain elongation.<sup>16</sup> There is strong evidence that ubiquilin-1 also stabilizes ubiquitinated substrates, preventing proteasomal degradation,<sup>21-24</sup> but the significance of this finding is not entirely clear. It is also

possible that increased ubiquitination of substrates by UBL/UBA domain proteins is not due to stabilization of ubiquitinated species, but rather to an active recruitment of E3 ligases to substrates, as is the case for the ubiquilin homolog KPC2.<sup>25</sup>

### Ubiquilin-1 is a Regulator of Membrane Protein Trafficking

Although ubiquitination is a well-established regulator of endocytosis and protein trafficking from the Golgi apparatus,<sup>26</sup> very little is known about the role of UBA/UBL domain proteins in this process. One prominent exception is ubiquilin-1, which regulates the trafficking of multiple transmembrane proteins. Ubiquilin-1 increases polyubiquitinated species of GABA receptors, and, similar to other UBA/UBL substrates, stabilizes them.<sup>24</sup> Interestingly, GABA receptor stabilization occurs in early secretory compartments, increasing their availability for rapid movement to the plasma membrane as a result of neuronal activity. Ubiquilin also negatively regulates stimulus-dependent G protein coupled receptor endocytosis.<sup>27</sup> This effect is mediated by the UBL domain binding to the cargo binding proteins epsins 1, 2 and Eps15 of the clathrin-mediated endocytosis pathway,<sup>27</sup> but the role of receptor ubiquitination has not been investigated. Ubiquilin-1 has also been shown to associate with multiple E3 ligases to promote ubiquitination and stabilization of substrates, including E6AP and  $\beta$ TRCP.<sup>23</sup> Significant questions remain regarding these studies, including (1) why ubiquilin-1 stimulated ubiquitination of substrates results in substrate stabilization, (2) the precise nature of the polyubiquitin chains and (3) how ubiquilin is regulating trafficking to different subcellular compartments in mechanistic detail.

### Ubiquilin-1 Regulates APP Trafficking through Non-Degradative Ubiquitination

APP maturation is known to be regulated by ubiquilin-1.<sup>28,29</sup> APP matures through the secretory pathway, where it undergoes classical *N*- and *O*-linked glycosylation and homodimerization as it transits

through the endoplasmic reticulum and Golgi apparatus.<sup>30,32</sup> After transport to the plasma membrane, APP can be recycled through the endocytic pathway.<sup>33</sup> In some cell types, up to 70% of APP is degraded by lysosomes, although it is unclear if degradation first requires transport to the plasma membrane.<sup>30,34,35</sup> Regardless, APP half-life is very short (less than 1 h) and the fraction of total APP protein present at the plasma membrane is minimal.<sup>34,36</sup> Almost nothing is known about the proteasomal degradation of APP. However, ubiquitination of APP may be partially mediated by the bifunctional chaperone/E3 ubiquitin ligase CHIP.<sup>37</sup>

Various proteins regulate the trafficking of APP to different subcellular compartments and thus regulate proteolytic processing. SorLA or LR11 acts as a sorting receptor that sequesters APP in the Golgi apparatus and reduces secretase processing of APP by limiting access to the secretase.<sup>38</sup> Numerous other proteins bind to the AICD to regulate vesicular trafficking and endocytosis of APP, notably X11 $\alpha$ /Fe65.<sup>33,36,39</sup>

Ubiquilin-1 knockdown accelerates APP maturation and increases amyloidogenic processing.<sup>29</sup> Our group and others have shown that ubiquilin-1 binds to APP.<sup>29,34</sup> As stated above, ubiquilin-1 binds to APP and exerts chaperone activity on the molecule, most likely via its Sti1 domains.<sup>9</sup> More recently, we have shown that ubiquilin-1 inhibits the exit of APP from the Golgi apparatus in a very similar fashion to SorLA.<sup>38</sup> Expression of ubiquilin-1 stimulates K63-linked polyubiquitination of lysine 688 (K688) in the AICD. K688 polyubiquitination results in decreased transport of APP to the plasma membrane, diminished amyloidogenic processing and reduced cell death.<sup>9,34</sup> Importantly, mutation of K688 to arginine in the AICD abrogated ubiquilin-1 dependent ubiquitination and Golgi sequestration,<sup>34</sup> providing strong evidence that ubiquitination of a single lysine residue in the AICD of APP is necessary for the observed effects on APP trafficking. Presumably, ubiquilin-1 recruits a yet unidentified E3 ligase to APP. Candidates include E3 ligases known to bind ubiquilin-1, such as HRD1,<sup>40</sup> E6AP<sup>23</sup> and  $\beta$ TRCP.<sup>23</sup>

Importantly, how K63-linked ubiquitination of APP results in decreased trafficking out of the Golgi apparatus is not readily apparent. One mechanism by which secretory proteins exit the Golgi apparatus is by binding to adaptor proteins that couple cargo to the vesicular transport machinery. It has recently been shown for other substrates that K63-linked polyubiquitin chains are a sorting signal from the Golgi.<sup>41</sup> The adaptor protein optineurin is a cargo adaptor<sup>42,43</sup> that binds K63-linked polyubiquitin chains,<sup>44</sup> and thus constitutes an attractive target for further investigation. K688 is positioned C-terminally only one amino acid away from the critical YENPTY sequence (single amino acid code) known to mediate APP sorting and endocytosis by binding to proteins such as X11 $\alpha$ /Fe65.<sup>45-48</sup> Thus, it is possible that polyubiquitination of K688 results in a steric barrier regulating the binding of these proteins critical for APP trafficking.

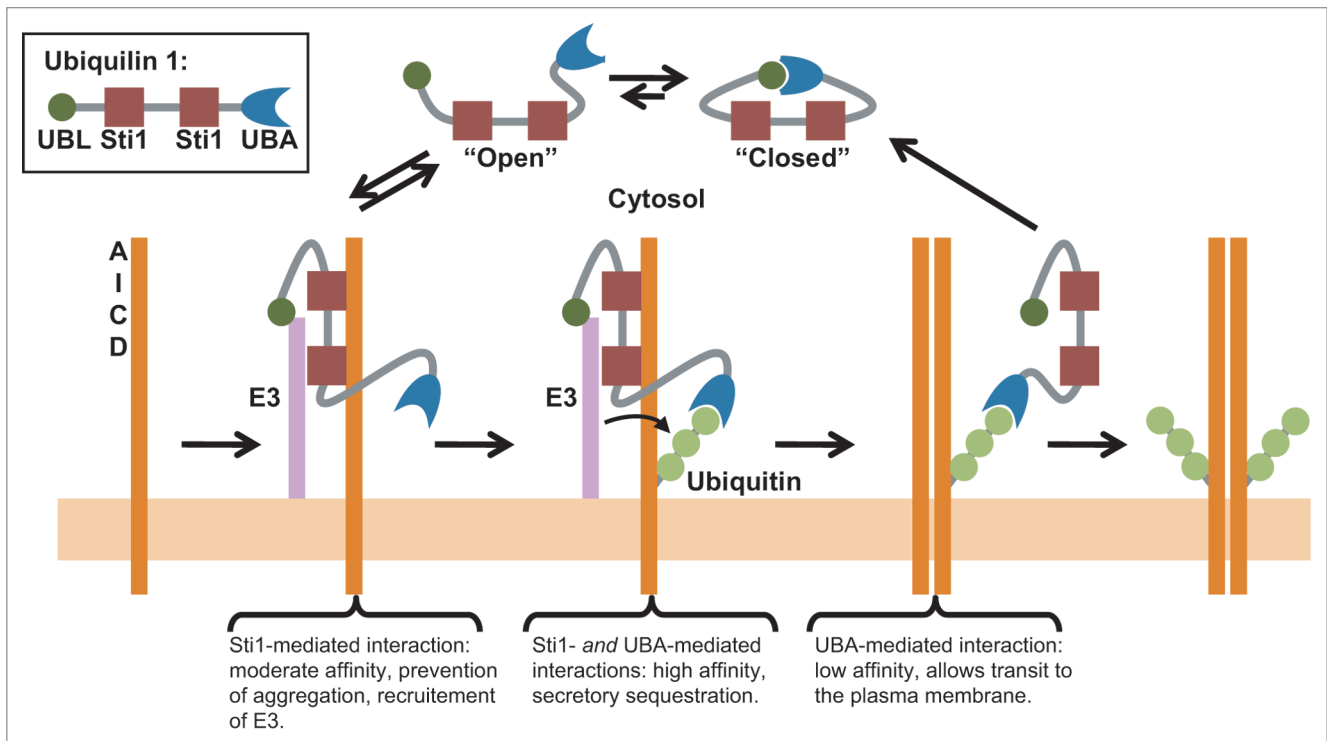
### Ubiquilin-1 as a Regulator of Protein Degradation

Ubiquilin-1 is involved in the regulation of other quality control pathways such as the ER-associated degradation (ERAD) pathway and autophagy. Ubiquilin-1 forms a complex with the ERAD protein ERASIN and the molecular chaperone p97/VCP to help clear misfolded proteins from the ER and deliver them to the proteasome.<sup>49</sup> Ubiquilin-1 also interacts with the ER stress protein Herp to enhance ER-associated degradation of certain substrates.<sup>40</sup> APP is synthesized and *N*-glycosylated in the ER, and misfolded APP may be degraded through the ERAD system before maturation through the Golgi apparatus and transport to the cell surface. Furthermore, APP has been shown to interact with the ER luminal chaperone BiP/ GRP78 and the stress-responsive chaperone/protease HtrA2/Omi.<sup>50</sup> A subpopulation of HtrA2 localizes to the cytosolic side of the ER membrane where it contributes to ERAD-mediated degradation of APP.<sup>50</sup> It is thus logical that ubiquilin-1 may contribute to ERAD of immature APP, however our overexpression studies indicates that ubiquilin-1 stabilizes this form of APP.<sup>34</sup>

Future studies will be needed to elucidate the effects of ubiquilin-1 on ERAD of APP. In addition to ERAD, ubiquilin-1 has an established role in the regulation of aggresome formation,<sup>51</sup> targeting presenilins to aggresomes,<sup>52</sup> is co-localized with various autophagy markers,<sup>53,54</sup> and is itself degraded by chaperone-mediated autophagy.<sup>55,56</sup> Thus, ubiquilin-1 has a central role in regulating protein trafficking, accumulation and degradation of diverse proteins relevant to neurodegenerative disease.

### A Model for Ubiquilin-1 as a Critical Regulator of APP Proteostasis

Our current model for ubiquilin-1 function is depicted in **Figure 1**. We postulate that first ubiquilin-1 binds to the AICD via the Sti-1 domain(s). This stabilizes an open conformation of ubiquilin-1 that results in an interaction of moderate affinity allowing binding and release events (consistent with a chaperone/client interaction). This prevents aggregation of APP<sup>28</sup> but allows subsequent maturation events. Next, ubiquilin-1 promotes ubiquitination of AICD, possibly by directly recruiting an E3 ligase. Ubiquilin-1 now binds to AICD via both the Sti1 domain(s) and UBA domains. This results in a high affinity interaction, which prevents the interaction of APP with the Golgi export machinery,<sup>32,57,58</sup> thus sequestering it in this part of the secretory pathway. Finally, conformational maturation (acquisition of appropriate tertiary and quaternary structure of APP, e.g., dimer formation) results in displacement of Sti-1 domains, thus resulting in a weakening of the interaction and eventual release. This could be a simple result of two APP protomers coming together and sterically hindering the Sti1 binding site and/or a conformational change on the protomer. Importantly, we have shown that ubiquilin-1 expression inhibits secretase/proteasomal degradation of APP without affecting lysosomal degradation.<sup>34</sup> Thus, ubiquilin-1 expression would function to increase net proteolysis of APP by lysosomal pathways. The end result is increased APP quality control, enhanced APP retention in the Golgi apparatus, increased lysosomal



**Figure 1.** Schematic overview of the potential role of ubiquilin-1 in regulating APP maturation and trafficking. Ubiquilin-1 binds to APP via the Sti1 domains and possibly recruits an E3 ligase. Ubiquitination greatly increases the affinity of ubiquilin-1 through interactions with the UBA domain. Maturation of APP decreases the affinity of ubiquilin-1 for APP leading to eventual release. See text for details.

degradation and ultimately decreased secretase processing.

## Conclusions

Our results suggest that ubiquilin-1 is a critical component of a trafficking checkpoint within the secretory pathway that prevents excessive amyloidogenic processing of APP by limiting access to secretase enzymes.<sup>34</sup> Consistent with this notion,

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we have shown that ubiquilin-1 overexpression decreases the A $\beta$ 42/40 ratio and prevents APP-induced toxicity in cells, while ubiquilin-1 knockdown exacerbates APP-induced toxicity.<sup>28</sup> A similar scenario may happen in AD patients, as ubiquilin-1 protein levels are inversely correlated with the Braak staging of the disease,<sup>28</sup> suggesting that therapies which restore ubiquilin-1 expression in the brain may represent a potential treatment for AD.

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## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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