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Commentary
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Ubiquilin Proteins are Critical Adaptors that Regulate Proteostasis

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Commentary

Our study 'The STI and UBA Domains of UBQLN1 are Critical Determinants of Substrate Interaction and Proteostasis', highlights the role of individual domains of UBQLN1 in stabilizing substrates (BCLb, IGF1R and ESYT2). Research on UBQLN1 has mostly been focused on its role in protein quality control and degradation of substrates that it binds to. However, we have identified substrates of UBQLN1 that are stabilized as a result of their association. We demonstrated that fate of substrates that UBQLN1 binds to is interaction-domain specific. The STI and UBA domains of UBQLN1 interact with BCLb, IGF1R and ESYT2 and stabilize these proteins while the stability of PSMD4 and BAG6, which interact with the UBL domain of UBQLN1 were not altered.

Introduction

The Ubiquilin family of proteins consists of 5 paralogous members (Figure 1A). All UBQLN proteins are similar in sequence and have 3 main structural domains, N-terminal UBL domain (ubiquitin-like), C-terminal UBA domain (ubiquitin-associated) and the STI chaperonin like regions in the middle (Figure 1B). UBQLN2 has an additional collagen-like triple helix region abutting the fourth STI domain. Mutations in this region have been associated with familial cases of Amyotropic Lateral Sclerosis and Frontotemporal dementia [1].

A

Gene	Location	Sequence similarity to UBQLN1 (%)
UBQLN1	9q21.32	100
UBQLN2	Xp11.21	74
UBQLN3	11p15.4	48
UBQLN4	1q22	60
UBQLNL	11	36

B

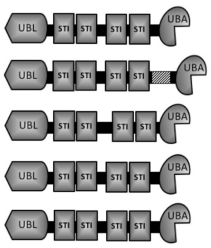

Figure 1: Ubiquilin family of proteins. A: There are 5 Ubiquilin proteins encoded by 5 different genes and by sequence and structure are similar to each other B: All Ubiquilin proteins have an N-terminal UBL domain (ubiquitin-like), C-terminal UBA domain (ubiquitin-associated) and STI regions in the middle. Ubiquilin2 has an additional collagen-like domain between the fourth STI and UBA domains.

Ubiquilin1 is an approximately 62 kDa protein and is the prototypical member of the UBQLN family. Ubiquilin1 is ubiquitously expressed in all tissues of the body. Within the cell, it is distributed throughout the cytoplasm, including at the plasma membrane but is distinctly absent from nuclei and vacuolar compartments (Figure 2A and 2B).

Ubiquilin1 is involved in diverse cellular processes from ERAD (endoplasmic reticulum associated degradation) [2,3] to autophagy [4-6] to apoptosis [7] to epithelial to mesenchymal transition (EMT) [2,8] (Figure 3A and 3B). Ubiquilin1 also interacts with a variety of substrates – proteins involved in the proteasomal machinery (PSMD4, BAG6) [9], cell surface receptors (GABA-A [10] , GPCR's [11], PSEN1/2 [12,13], IGF1R [9]), transcription factor regulators (IκBα) [14] and other transmembrane proteins ESYT2 [9], CD47 [15], BCLb [16]. Considering the range of substrates and cellular processes Ubiquilin1 is involved in, we hypothesize that Ubiquilin1 is a versatile, multi-purpose adaptor, housing domains for binding to other proteins leading to functional specificity of resulting multimeric complexes. Our study investigated the role of individual domains of Ubiquilin1 in determining outcomes of substrate-Ubiquilin1 interaction.

Role of Ubiquilin Proteins in Cancer

Ubiquilin proteins have mostly been studied in the field of neurodegenerative disorders. Recently, a new interest has developed in Ubiquilin's role in cancer. Data published on Ubiquilin proteins in the field of cancer has been inconsistent so far. UBQLN1 was reported to be lost in approximately fifty percent of non-small cell lung adenocarcinomas [2]. siRNA mediated loss of UBQLN1 and mir155 mediated downregulation of UBQLN1 in lung cancer cells has been shown to promote an EMT-like phenotype. It has been shown that downregulation of UBQLN1 leads to a significant increase in mesenchymal markers, including Vimentin, Snail and ZEB1 and therefore, UBQLN1 has been suggested to play a role in suppression of metastasis in lung cancer [8]. However, overexpression of UBQLN1 in lung cancer cells [17] and UBQLN2 in osteosarcomas [18] have also been reported. At the protein level, UBQLN1 stabilizes tumor suppressor p53 [14,19] as well as anti-apoptotic protein BCLb [9,16]. Thus, it is fallible to conclude a definitive role of UBQLN proteins in cancer development until large sample sizes and deeper stratification of patients and subtypes of cancers have been investigated. Equally important is to understand how Ubiquilin1 differentially regulates multiple substrates thus providing a clearer insight into Ubiquilin related diverse findings in cancers.

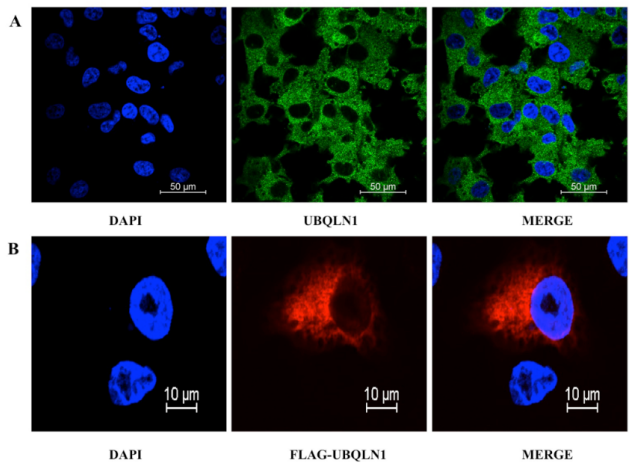

Figure 2: Cellular distribution of Ubiquilin1. Confocal microscopy images of indirect immunofluorescence staining of endogenous UBQLN1 (green) in normal lung epithelial HPL1D cells (A) and over-expressed FLAG tagged UBQLN1 (red) in HeLa cells (B). Both endogenous and exogenous UBQLN1 are ubiquitously expressed in the cytosol. However, in both these conditions, UBQLN1 is distinctly absent from nuclei and vacuolar compartments.

A

UBQLN facilitates	
Degradation	**Stabilization**
Ataxin3 (28)	PSEN1/2 (13)
EPS15 (28)	BCLb (16)
NS5B (29)	IGF1R (9)
HSJ1a (28)	ESYT2 (9)
TDP43 (30)	IκBα (19)
	GABA-A (31)
	p53 (14,19)

B

Potential Roles
Protein quality control (32-34)
Endoplasmic Reticulum Associated Degradation (2,3)
Autophagy (4-6)
Apoptosis (7)
Receptor Trafficking (35,36)
Epithelial to Mesenchymal Transition (2,8,37)

Figure 3: Functions of Ubiquilin. A: List of published substrates that UBQLN1 facilitates degradation or stabilization of A taxin3: a deubiquitinase enzyme, EPS15: Epidermal growth factor receptor substrate 15, NS5B: Nonstructural protein 5B is a RNA polymerase found in Hepatitis C virus, HSJ1a: Homo sapiens J domain protein (HSJ1) is a J-domain containing co-chaperone and facilitates proteasomal degradation of ubiquitinated substrates, TDP43: TAR DNA binding protein 43, PSEN1/2: Presenilin 1 and Presenilin2, BCLb: anti apoptotic BCL2-like protein, IGF1R: Insulin-like Growth Factor 1 Rceptor, ESYT2: Extended Synaptotagmin 2, IκBα: member of family of proteins that inhibit NFκB activity, GABA-A: ligand gated ion channel receptors that function as major inhibitory neurotransmitters, p53: tumor suppressor protein B: List of published cellular processes that UBQLN1 may regulate.

Proteostasis of Transmembrane Substrates of Ubiquilin1

We demonstrated that Ubiquilin1 regulates three transmembrane proteins – BClb, an anti-apoptotic mitochondrial membrane protein, insulin-like growth factor 1 receptor (IGF1R), a cell surface receptor tyrosine kinase and extended synaptotagmin 2 (ESYT2), a calcium sensing protein localized at ER-plasma membrane contact sites. The STI domains of UBQLN1 are essential for interaction with these substrates while the UBA domain is required to protect them from degradation.

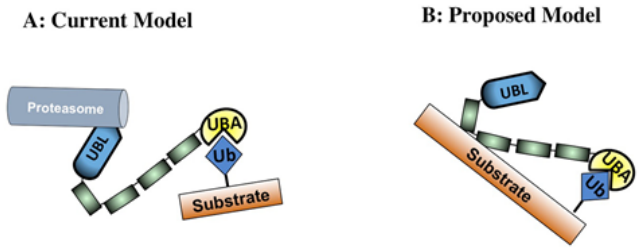

Figure 4: Working model of Ubiquilin1. A: Current working model of UBQLN1 hypothesizes that the UBA domain of UBQLN1 interacts with substrates while the UBL domain simultaneously interacts with the S5a cap of the proteasome facilitating degradation of the associated substrate B: Proposed 'substrate stabilization model': Primary association of UBQLN1 with substrate occurs through the STI domains and a secondary association through its UBA domain, which leads to stabilization of the substrate.

We used BCLb as a model substrate to conduct our experiments. Ubiquilin1 interacts with BCLbWT (wild-type BCLb) and BCLbK0 (a construct of BCLb with all four of its Lysines mutated to Arginine), indicating that Ubiquilin1 associates with ubiquitinated and non-ubiquitinated BCLb. However, Ubiquilin1 is unable to interact with BCLbΔTM (missing the transmembrane domain) implying that the transmembrane domain of BCLb is essential to be recognized and therefore be regulated by Ubiquilin1. Upon exposure to a translational inhibitor (cycloheximide), BCLbWT gets degraded in 16 hours. However, in the presence of UBQLN1, its life is prolonged. UBQLN1ΔUBA (missing UBA domain) is unable to prevent cycloheximide-induced degradation suggesting that the UBA domain is critical in stabilizing BCLbWT. We have previously shown that the lysine residues on BCLb get mono-ubiquitinated [9,16] and van de Kooji et al. demonstrated that the K128 residue on BCLb can form K48 poly-ubiquitin chains which signals BCLb for proteasomal degradation [20]. We hypothesize that the UBA domain of UBQLN1 acts as a ubiquitin receptor, prevents formation of K48 poly-ubiquitin chains on K128 residue of BCLb, thus averting its signaling for proteasomal degradation.

IGF1R is a cell surface receptor tyrosine kinase and upon ligand binding, it gets activated followed by subsequent intracellular trafficking [21]. Our IP/MS and IP/WB data indicate that Ubiquilin1 interacts with IGF1R and plays a role in its stability. Like with BCLb, the STI domains are responsible for interaction with IGF1R and the UBA domain is critical in protecting it from MG132 (proteasomal inhibitor) induced degradation. Detailed analyses of association of IGF1R and UBQLN1 were not performed for this manuscript;

however, testing conditions under which UBQLN1 interacts with IGF1R are warranted. It will be interesting to determine whether Ubiquilin1 can alter trafficking, synthesis, overall expression or activity of IGF1R. Previously, Ubiquilin2 has been shown to negatively regulate clathrin-mediated endocytosis of G Protein-Coupled Receptors (GPCR's) [11]. The UBL domain of Ubiquilin1 interacts with the UIMs (ubiquitin interacting motif) of EPS15, Hrs, Hbp [22]. These proteins form a multi-complex and assist in sorting of ubiquitinated cargo (endocytosed EGF receptor) into multi-vesicular bodies [23]. Whether Ubiquilin1 participates in a similar complex for regulation of IGF1R is not known. As IGF1R is involved in processes of normal growth, development, metabolism and even cancer progression, understanding its regulation by Ubiquilin1 can be of tremendous value to many disciplines.

There are three Extended Synaptotagmin proteins (ESYT1/2/3). ESYTs have an N-terminal ER-membrane binding domain, a mitochondrial-lipid-binding protein domain (SMP), and multiple calcium sensing C2 domains [24]. Proteins like ESYTs connect the vast ER network to different compartments in the cell like plasma membrane and mitochondria and facilitate exchange of molecules and ions and regulate cell signaling between these compartments. ESYT2/3 are not integral membrane proteins like ESYT1, but are inserted into the ER membrane through its N-terminal transmembrane domain and with the plasma membrane through its three C2 domains. Recently, it was shown that ESYT2 interacts with activated Fibroblast Growth Factor Receptor (FGFR) [25]. Subsequently, ESYT2 interacts with early endosome marker EEA1 during the internalization phase of FGFR upon activation with FGF. Additionally, ESYT2 interacts with AP2, another protein involved in clathrin-mediated endocytosis [26]. Our data demonstrate that UBQLN1 strongly interacts with ESYT1 and ESYT2 but not with ESYT3 (Immunoprecipitation/Mass Spectrometry). We confirmed that STI domains of UBQLN1 are critical in binding to ESYT2 (Immunoprecipitation/Western Blot) and the UBA domain protects it from MG132 induced degradation [9].

Ubiquilin1 may play an important role in endocytosis and trafficking of proteins. Whether this role is *via* its UBA domain associating with ubiquitin on substrates or through one of its other domains remains to be investigated.

Current Working Model of Ubiquilin1

Over the years, Ubiquilin1 has been shown to positively and negatively regulate a variety of proteins (Figure 3). Several studies hypothesize that UBL mediated interactions of Ubiquilin1 with its substrates result in their proteasomal degradation. We demonstrated that STI and UBA mediated interactions of Ubiquilin1 result in proteostasis of three transmembrane proteins such that primary association of UBQLN1 with substrate occurs through the STI domains and a secondary association through its UBA domain leads to stabilization of the substrate (Figure 4A and 4B). Additionally, we showed that the UBA domain of Ubiquilin1 binds non-preferentially to different ubiquitin linkages, Ubiquilin1 dimerizes through its STI-4 domains and Ubiquilin1 continues to interact with its substrates in its monomeric and dimerized forms [9]. Most importantly, we demonstrated that Ubiquilin1 may be a key regulator of transmembrane proteins like BCLb, IGF1R and ESYT2 and potentially influence their activity. So far, we have only scratched the surface of understanding the mechanics of interaction between these proteins. However, detailed experiments characterizing their regulation are warranted. For example, for a transmembrane protein like IGF1R that gets internalized upon ligand binding, pulse chase assays, endocytic compartment colocalization studies, recycling and degradation kinetics in the presence and absence of UBQLN1 will provide useful information on role of UBQLN1 in receptor trafficking. ESYT proteins are calcium sensing proteins present at ER-plasma membrane contact sites and participate in connecting cellular compartments [27-32] UBQLN1 interacts with several transmembrane proteins which directly or indirectly interact with cytoskeletal proteins like Vimentin [15]. We hypothesize that UBQLN1 acts as a scaffold to hold protein complexes together and may also play a role in preserving normal cellular morphology and signaling networks [33-37]. Identifying interactions of UBQLN1 with components of the membrane and cytoskeleton using co-immunoprecipitation analysis, crosslinking protein interaction assays for detection of transient interactions, visualization of contact sites and tracking changes in live cells *via* immunofluorescence and electron microscopy will be valuable.

Conclusion

We conclude that Ubiquilin1 performs a variety of functions in cells and fate of substrates it binds to is interaction-domain specific. Individual domains may determine Ubiquilin1's underlying mechanism of action and elucidate its role in neurodegenerative disorders as well as in cancers.

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