

Ubiquilin 2: Shuttling Clients Out of Phase?

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Ubiquilin 2 (UBQLN2) is an amyotrophic lateral sclerosis-linked molecular chaperone with a prion-like domain that directly engages ubiquitin to triage clients for proteasomal degradation. Dao et al. (2018) now establish that UBQLN2 forms ubiquitin-labile liquids, which may enable UBQLN2 to specifically extract ubiquitylated clients from stress granules for degradation.

In response to stress, cells implement several damage-limitation programs, including the biogenesis of cytoplasmic stress granules (SGs) (Protter and Parker, 2016). These dynamic membraneless organelles assemble upon inhibition of translation initiation to protect their protein and RNA constituents during deleterious periods. Once stress ebbs, SGs resolve to liberate their proteins and RNAs, which may resume their functions or be degraded. SGs assemble via liquid-liquid phase separation (LLPS) driven by multivalent RNA-RNA, RNA-protein, and protein-protein interactions, including multiple transient contacts between low-complexity, prion-like domains (PrLDs) (March et al., 2016; Protter and Parker, 2016). Excessive SG assembly or defective SG dissolution after stress is linked with neurodegeneration and may reflect altered phase behavior of SG-resident RNA-binding proteins (RBPs) or altered protein-degradation programs (March et al., 2016). Ubiquilins (UBQLNs) are a family of four (-1, -2, -3, and -4) paralogous molecular chaperones that shuttle ubiquitylated clients to the proteasome for degradation (Hjerpe et al., 2016; Itakura et al., 2016). Mutations in UBQLN2 or UBQLN4 that impair protein degradation cause amyotrophic lateral sclerosis (ALS) (Deng et al., 2011; Edens et al., 2017). However, the mechanisms underlying the complete functional repertoire of UBQLNs are not understood. In this issue of *Molecular Cell*, Castañeda and coworkers determine that UBQLN2 undergoes LLPS and accumulates in SGs (Dao et al., 2018). Ubiquitin or polyubiquitin abolishes UBQLN2 LLPS and may enable UBQLN2 to extract ubiquitylated clients from SGs for degradation (Dao et al., 2018).

Dao et al. establish that UBQLN2 is diffusely localized in the cytoplasm, but accumulates in SGs under a variety of stresses (Dao et al., 2018). Importantly, purified UBQLN2 undergoes reversible LLPS *in vitro* under relatively physiological conditions, which likely enables UBQLN2 recruitment to SGs *in vivo* (Dao et al., 2018). To map the domain requirements for UBQLN2 LLPS, several UBQLN2 deletion constructs were assessed for propensity to form liquid drops. Like other UBQLNs, UBQLN2 harbors a ubiquitin-like domain (UBL; residues 33–103) that associates with proteasomal subunits, two STI1 domains (STI1-I, residues 178–247, and STI1-II, residues 379–462) that interact with molecular chaperones including Hsp70, and a ubiquitin-associating domain (UBA, residues 577–620) that binds to ubiquitin (Figure 1A) (Ko et al., 2004). UBQLN2 also contains a proline-rich region (Pxx) (residues 491–538), which is not found in other UBQLNs. Like UBQLN1, UBQLN2 harbors a PrLD (residues 339–462), which overlaps with STI1-II (Figure 1A) (March et al., 2016). PrLDs are low-complexity domains with a distinctive amino acid composition enriched in glycine and uncharged polar residues such as glutamine, asparagine, tyrosine, and serine, which resemble prion domains that enable various yeast proteins to form prions (March et al., 2016). PrLDs also drive functional LLPS and deleterious aggregation of several ALS-linked RBPs such as TDP-43 and FUS (Figure 1A) (March et al., 2016). Dao et al. show that UBQLN2 450–624, which includes a portion of the PrLD, STI1-II, Pxx, and UBA domains, is the minimal region necessary for LLPS *in vitro* and accumulation in SGs *in vivo* (Dao et al., 2018). NMR revealed this region to be largely disordered.

Indeed, the UBA domain is folded, but residues 487–580 are intrinsically disordered with backbone relaxation parameters akin to the FUS PrLD (Dao et al., 2018). However, robust UBQLN2 LLPS under diverse conditions requires the STI1-II portion of the PrLD (Dao et al., 2018). Deletion of the STI1-II portion of the PrLD or the UBA domain abolishes UBQLN2 LLPS *in vitro* (Dao et al., 2018). Thus, several UBQLN2 domains engage in multivalent interactions that elicit LLPS, including major contributions from the STI1-II portion of the PrLD and the UBA domain.

To shuttle ubiquitylated clients to the proteasome, UBQLNs bind ubiquitin and proteasomes directly (Itakura et al., 2016; Ko et al., 2004). Remarkably, UBQLN2 binding to ubiquitin or polyubiquitin *in vitro* disrupted UBQLN2 LLPS. Indeed, UBQLN2 LLPS was eliminated by monoubiquitin and K48-linked di- and tetra-ubiquitin chains, a ubiquitin linkage associated with proteasomal degradation. Specific binding of ubiquitin to the UBA domain disrupts multivalent interactions that maintain UBQLN2 liquids (Dao et al., 2018). Thus, the UBA domain acts as a regulatory switch for UBQLN2 LLPS. As many proteins are ubiquitylated in SGs, UBQLN2 may bind to these ubiquitin chains inside the SG and subsequently disassociate from the SG phase to ferry substrates to the proteasome (Figure 1B) (Dao et al., 2018). While Dao et al. focused on K48-linked ubiquitin chains, it will also be interesting to consider other ubiquitin linkages. Given that UBQLNs are also connected to macroautophagy and interact with LC3, an autophagosome marker (Rothenberg et al., 2010), perhaps UBQLN2 operates in a similar manner with alternative ubiquitin linkages to target clients for autophagy.



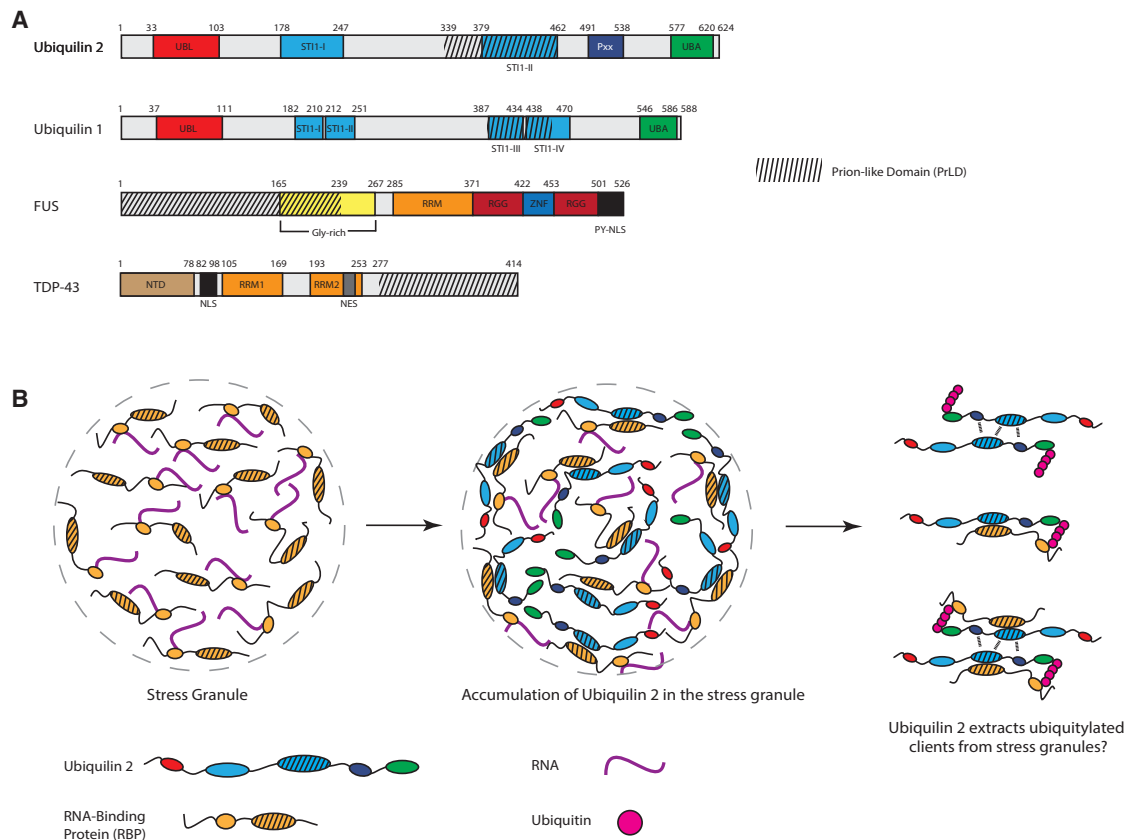


Figure 1. UBQLN2 May Extract Ubiquitylated Clients from SGs

(A) Domain architecture of UBQLN1, UBQLN2, FUS, and TDP-43. The position of various domains including the PrLD is indicated.

(B) *In vivo*, UBQLN2 LLPS enables UBQLN2 accumulation in SGs. Interactions between the PrLD of UBQLN2 and PrLDs of SG-resident RBPs may help fluidize the SG. Interactions between the UBA domain of UBQLN2 and ubiquitylated clients reverse UBQLN2 LLPS, which may enable UBQLN2 to shuttle clients out of SGs for proteasomal degradation.

Dao et al. advance a model in which UBQLN2 undergoes LLPS driven by multivalent interactions mediated by the STI1-II portion of the PrLD, the Pxx domain, and the UBA domain, which enables SG association *in vivo*. Upon binding to ubiquitin chains via its UBA domain, UBQLN2 LLPS is disrupted, which elicits release of client-bound UBQLN2 from the SG to enable client degradation by the proteasome (Figure 1B). This model provides a mechanism by which UBQLN2 may extract ubiquitylated, ALS-linked RBPs such as TDP-43 and FUS from SGs (Figure 1B) (Dao et al., 2018). Moreover, interactions between the UBQLN2 PrLD and PrLDs of TDP-43 and FUS may fluidize SGs and prevent aberrant phase transitions to pathological aggregates (March et al., 2016). These proposed UBQLN2 activities could have therapeutic utility and are likely corrupted in disease. Indeed,

UBQLN2 co-localizes with cytoplasmic inclusions in a wide variety of ALS cases (Deng et al., 2011). However, these inclusions could be resolved with therapeutic protein disaggregases (Shorter, 2017). Moving forward, it will be critical to confirm whether UBQLN2 engages ubiquitylated clients inside SGs and removes them from the SG phase for proteasomal degradation *in vivo*. It will also be important to determine the LLPS propensity of ALS-linked UBQLN2 mutations, the majority of which cluster near the proline-rich Pxx region (Deng et al., 2011). Finally, it will be enlightening to learn whether ALS-linked UBQLN2 variants are defective in ubiquitylated client extraction from SGs.

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No Longer Hidden Secrets of Proton Pumping: The Resolution Revolution Enlightens V-ATPases

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In this issue of *Molecular Cell*, Roh et al. (2018) present a high-resolution cryo-EM structure of the nanodisc-reconstituted yeast V_o proton channel that provides important new insights into subunit arrangements and the proton translocation pathway in V-type ATPases.

The rotary ATPase family comprises the mitochondrial ATP synthases (F-ATPases), vacuolar ATPases (V-ATPases), and archaeal ATPases (A-ATPases), all of which utilize either a proton gradient to produce ATP or ATP hydrolysis to generate a proton gradient (Muench et al., 2011). These sophisticated enzymes are crucial players in many vital cellular processes, and mutations affecting their functions cause a large variety of human diseases. An important member of this family, the V-ATPase, consists of an ATP-hydrolyzing V_1 domain composed of the subunits A_3 , B_3 , E_3 , F , G_3 , C , H , and D that are linked to a membrane-bound V_o domain consisting of a variable-sized ring of c subunits, subunits a and e , and the recently identified subunit f (Figure 1). Through ATP hydrolysis in the V_1 domain, the central rotor axle is rotated by $\sim 120^\circ$ per ATP used. The resulting torque drives the rotation of the linked c -ring against subunit a , resulting in proton translocation across the membrane and the acidification of a subcellular compartment. For many years, the fundamental mecha-

nisms of torque generation and ATP handling have been established with crystal structures of the F_1 , V_1 , and A_1 domains revealing the structural rearrangements that accompany ATP binding and hydrolysis (Suzuki et al., 2016). However, due to limitations in resolution, the exact pathway of proton transport within the membrane-bound V_o domain has remained largely elusive. In this issue of *Molecular Cell*, Roh et al. (2018) present a high-resolution cryo-electron microscopy (cryo-EM) structure of the yeast V_o proton channel that sheds exciting new light on the mechanism of proton pumping by V-ATPases.

The first single-particle cryo-EM reconstruction of the V-ATPase was published in 2009 to a modest ~ 20 Å resolution. However, recent developments in electron microscopy (EM) have enabled a more detailed understanding of the proton transport mechanism, including significantly higher-resolution structures of both the *Manduca sexta* (~ 9 Å) and the yeast V-ATPase (~ 7 Å) complex (Rawson et al., 2015; Zhao et al., 2015). Other structural studies have made use

of the fact that V-ATPases undergo controlled dissociation resulting in V_1 separating from the V_o domain such that ATP hydrolysis and proton transport are silenced (Oot et al., 2017). The individual domains have been determined to 6.2 Å and 3.9 Å resolution, respectively (Oot et al., 2016; Mazhab-Jafari et al., 2016). As the resolution of these models has increased, our understanding of how ATP hydrolysis is linked to proton movement over the membrane and how the complex resists the backflow of protons after the generation of a membrane potential has been developed. In 2015, a key step forward in understanding the mechanism of proton transport within the a subunit was taken, when it was shown in both the F- and V-ATPase that two horizontal α helices flank the c -ring facilitating proton transport. This finding was in stark contrast to earlier models, which reported an eight α -helical bundle with all helices traversing the membrane (Allegritti et al., 2015; Zhao et al., 2015).

In a new paper in this issue of *Molecular Cell*, the Wilkens group has now

