Only solid waste, please!

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Summary

To elucidate the mechanism driving selective autophagy of protein aggregates, or "aggrephagy," Ma et al. (2022) identify chaperonin TRiC subunit CCT2 as a receptor that specifically promotes the clearance of solid aggregates, but not liquid-like condensates in a ubiquitin-independent manner.

Main text

Protein aggregates are a hallmark of all neurodegenerative disorders and other human pathologies and are believed to cause damage to cells. Hence, pathways must be in place to recognize and remove protein aggregates. Autophagy is an important waste disposal pathway known to selectively deliver protein aggregates for lysosomal degradation, which is called "aggrephagy".

Other types of selective autophagy are known, such as mitophagy, xenophagy and lysophagy. These pathways usually involve ubiquitin-binding receptors (e.g. p62/SQSTM2, NBR1, NDP52, TAX1BP1, OPTN, Tollip) that harbor both a ubiquitin-binding domain (UBA), which recognizes ubiquitinated cargoes, and an LC3-interacting region (LIR), which binds to LC3 and GABARAP members of the human ATG8 family that are conjugated to nascent autophagosome membranes (Simonsen and Wollert, 2022). These receptors can also target ubiquitinated protein aggregates, albeit non-specifically.

How the autophagy machinery recognizes protein aggregates and selectively targets them for degradation is a major question in the field. Is there an "aggrephagy receptor" that specifically recognizes the misfolded or aggregated state of proteins and funnels such aberrant species into the autophagic waste disposal pathway? So far, dedicated aggrephagy receptors have been reported in yeast (Atg19) and *C.elegans* (e.g. SEPA-1); however, no aggrephagy receptor has been identified in mammalian cells. Intriguingly, the known aggrephagy receptors also seem to have a preference for liquid-like protein condensates rather than solid-like aggregates (Yamasaki et al., 2020; Zhang et al., 2018).

To hunt down a potential aggrephagy receptor, Ma et al. (2022) used an elegant *in vitro* reconstitution approach in permeabilized cells to quantify binding of fluorescently labelled LC3 with polyQ Huntingtin (Htt) aggregates. Following LC3-based sorting and aggregate isolation, quantitative mass spectrometry analyses identified a surprisingly large number of chaperones and co-chaperones enriched on LC3-positive aggregates. The most enriched chaperone was CCT2, a subunit of the chaperonin TRiC. CCT2 knockdown decreased LC3-aggregate association, whereas CCT2 overexpression enhanced this association. Ma et al. even confirmed these findings *in vivo* utilizing a mouse model of Huntington's disease, whereby adenovirus-mediated expression of CCT2 in the striatum reduced the levels of polyQ Htt aggregates. CCT2 was also recruited to other protein aggregates linked to neurodegenerative diseases, such as Tau P301L and SOD1 G93A aggregates, and specifically enhanced their clearance without altering bulk autophagic flux or mitophagy.

How does CCT2 bind to LC3 proteins in the absence of a classical LIR? Among the six different human ATG8 proteins (LC3A, LC3B, LC3C, GABARAP, GABARAPL1 and L2), CCT2 binds most strongly to LC3C *in vitro* via two triple consecutive hydrophobic motifs (VLL and VIL) in the C-terminal region of CCT2. While the preference of these motifs for binding LC3s/GABARAPs in cell remains to be determined, they are required for association with ATG8 proteins and for clearance of Htt aggregates

upon CCT2 overexpression. Moreover, they not only work as a LIRs in mammalian cells, but also in yeast. Notably, two CCT2 point mutations that cause the congenital retinopathy, Leber Congenital Amaurosis, display reduced LC3 association and impaired Htt aggregate degradation, suggesting that defects in CCT2-mediated aggrephagy may be relevant to retinopathy.

How does CCT2 recognize the protein aggregates? In contrast to p62 and other autophagy receptors, it does not possess a ubiquitin-binding UBA domain, so it is unlikely to recognize aggregates via their polyubiquitin marks. Indeed, Ma et al. found that CCT2 does not bind to polyubiquitin chains synthesized *in vitro* or from cell lysates and that it clears aggregates independent of known ubiquitin-binding receptors, such as p62, NBR1 and TAX1BP1, and independent of Hsp70, the key receptor of chaperone-mediated autophagy.

Next, Ma et al. interrogated whether CCT2 could distinguish protein aggregates by their biophysical nature. Solid aggregates, such as Htt polyQ and other inclusions found in neurodegenerative disorders (such as Tau and FUS aggregates), are believed to form through liquid-liquid phase separation and transition from a liquid-like state to solid-like condensates over time (Nedelsky and Taylor, 2019). The authors showed that lysosomal degradation of “young” (24h) liquid-like FUS condensates depends on the classical ubiquitin receptors p62, NBR and TAX1BP1, whereas disposal of “aged” (72h) solid-like condensates does not rely on this set of receptors, but on CCT2. A similar preference was observed in yeast.

What regulates this selectivity for aged deposits remains unclear. *In vitro* reconstitution experiments with purified FUS condensates suggest that no exogenous factors are required for the recognition of aggregates by CCT2, indicating that CCT2 may sense specific structural features in aging FUS condensates, such as short cross-beta sheets observed in the FUS fibril core (Murray et al., 2017). Of note, the authors mapped the critical aggregate-binding region of CCT2 to its apical domain, which is the same interface required for substrate recognition of the chaperonin and is functionally conserved across the eight other subunits of TRiC (CCT1-8) (Gestaut et al., 2019). Importantly, CCT2 alone is sufficient to promote clearance of Htt polyQ aggregates, as the authors show from experiments in cells depleted of CCT4 or CCT5.

Interestingly, the substrate recognition sites of TRiC subunits do not comprise hydrophobic binding interfaces as seen in other chaperones, but instead employ a series of biochemically diverse residues that may recognize topologically complex substrates with aggregation-prone, beta-sheet folds (Joachimiak et al., 2014). In this context, TRiC was shown to modulate aggregation of amyloid proteins, including Htt polyQ (Hipp et al., 2019).

What triggers CCT2 to dissociate from TRiC and function as an aggrephagy receptor? Ma et al. provided initial evidence that accumulation of Htt polyQ aggregates may disrupt TRiC and expose non-classical LIR motifs in CCT2 required for its interaction with LC3. Consistent with this notion, monomeric CCT2, but not fully assembled TRiC, was able to bind LC3. Conversely, conditions that re-establish TRiC’s integrity, such as overexpression of all its subunits together, reduced lysosomal Htt polyQ degradation.

To what extent this new aggrephagy receptor function is unique for CCT2 among the eight TRiC subunits is unclear. While the VLIR motifs do not seem to be conserved across the different TRiC components, ATG8 family binding of the other CCT proteins remains to be tested. Given that CCT1 overexpression has been shown to reduce Htt polyQ aggregation (Sontag et al., 2013), it could well be that the monomeric forms of other CCT proteins also retain the ability to bind aggregates and deliver them for lysosomal degradation. Whether additional chaperones, such as heat shock proteins, exert a similar moonlighting function in aggrephagy remains to be investigated. The findings by Ma et al. should spark new research into the solid vs. liquid waste disposal machinery of cells.

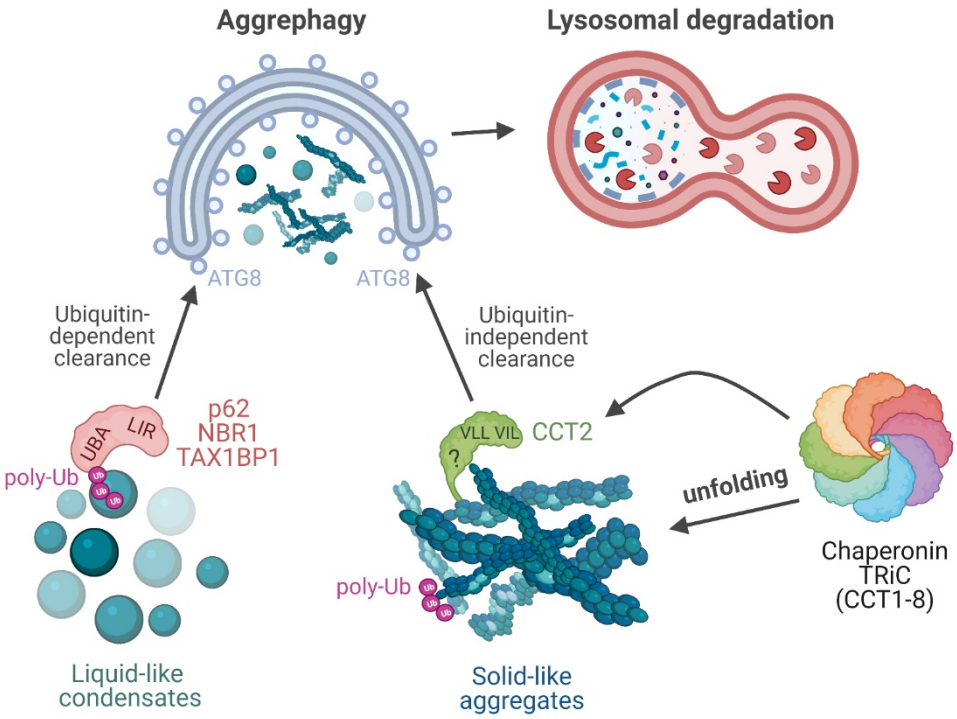

Figure: CCT2's dual role as chaperonin subunit and autophagy receptor for solid protein aggregates. A subunit of the chaperonin TRiC, CCT2, has an unexpected moonlighting function. CCT2 monomer acts as receptor for solid protein aggregates and targets them for autophagic degradation (aggrephagy). CCT2 does not harbor a ubiquitin binding domain and may bind beta-sheet folds in solid aggregates, but not liquid-like condensates, through an undefined mechanism. CCT2 possesses a non-classical hydrophobic LIR motif (VLL, VIL) that interacts with ATG8 family proteins on the autophagosome membrane. In contrast, liquid-like condensates are cleared with the help of ubiquitin-dependent autophagy receptors, e.g. p62/SQSTM1, NBR1 and TAX1BP1, that recognize ubiquitinated cargo via a UBA domain and bind to ATG8 family proteins via a LIR motif.

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Declaration of Interests

The authors declare no competing interests.

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