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Aggrephagy at a glance

Bernd Bauer[1,2], Sascha Martens[1,*] and Luca Ferrari[1,*]

ABSTRACT

Cells keep their proteome functional by the action of the proteostasis network, composed of the chaperones, the ubiquitin-proteasome system and autophagy. The decline of this network results in the accumulation of protein aggregates and is associated with aging and disease. In this Cell Science at a Glance and accompanying poster, we provide an overview of the molecular mechanisms of the removal of protein aggregates by a selective autophagy pathway, termed aggrephagy. We outline how aggrephagy is regulated by post-translational modifications and via auxiliary proteins. We further describe alternative aggrephagy pathways in physiology and their disruption in pathology. In particular, we discuss aggrephagy pathways in neurons and accumulation of protein aggregates in a wide range of diseases. Finally, we highlight strategies to reprogram aggrephagy to treat protein aggregation diseases.

KEY WORDS: Autophagy, Proteostasis network, Neurodegeneration

[1]Max Perutz Labs, University of Vienna, Vienna BioCenter, Dr Bohr-Gasse 9/5, 1030 Vienna, Austria. [2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Campus-Vienna-Biocenter 1, 1030 Vienna, Austria.

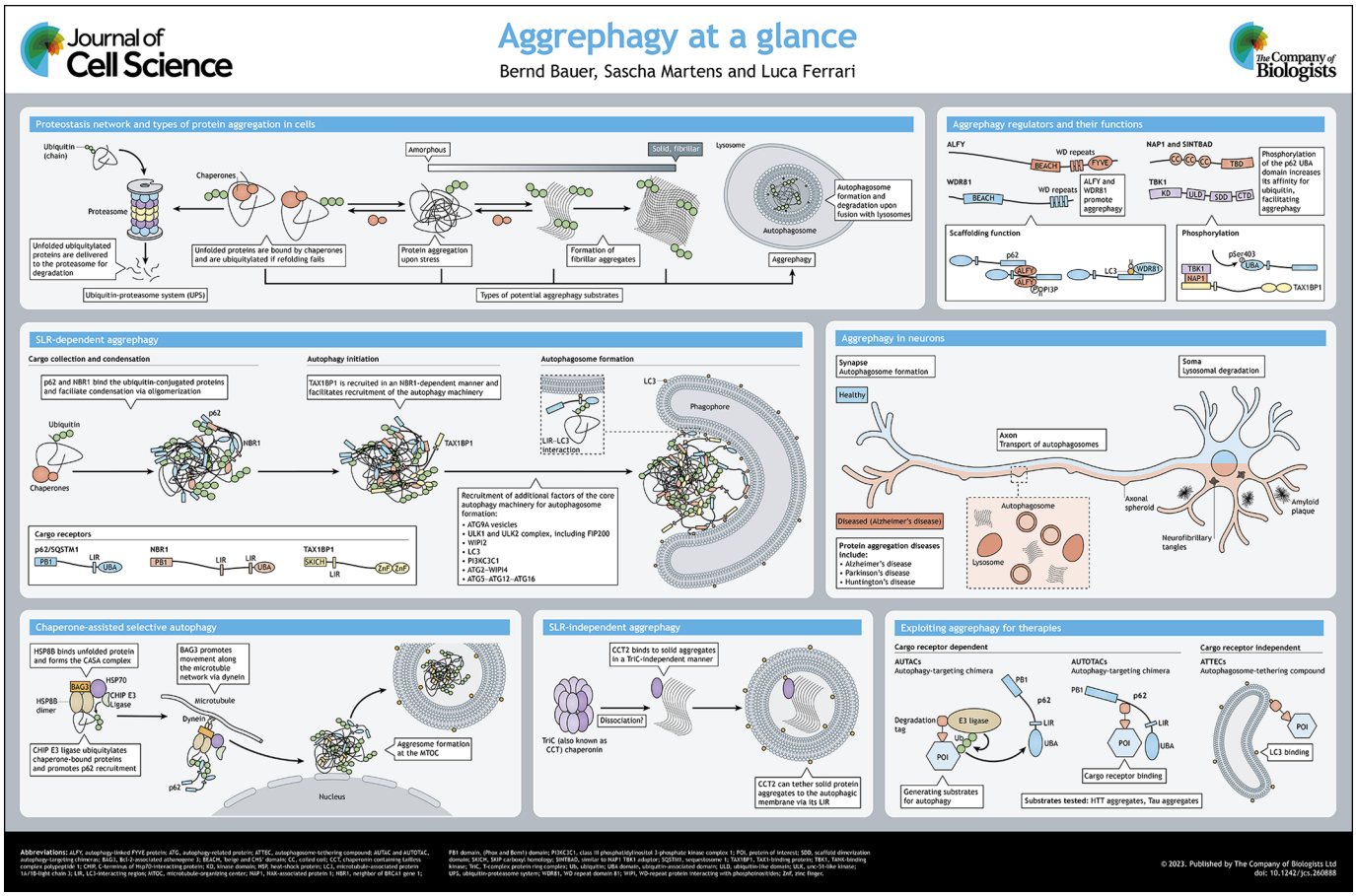
*Authors for correspondence (sascha.martens@univie.ac.at; luca.ferrari@univie.ac.at)

B.B., 0000-0002-2015-0212; S.M., 0000-0003-3786-8199; L.F., 0000-0003-1934-157X

Introduction

All living systems must keep their proteome functional by selectively degrading misfolded, aggregation-prone proteins. Uncontrolled protein aggregation is a major hallmark of many pathologies, including neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Hipp et al., 2019).

Eukaryotes evolved three main pathways to safeguard their proteome – the chaperone network, the ubiquitin-proteasome system (UPS) and autophagy. Collectively their activities comprise the highly integrated proteostasis network (Hipp et al., 2019).



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Protein misfolding and aggregation can be caused by external insults, such as heat shock, errors in protein biosynthesis and protein aging. To counteract protein aggregation, chaperones evolved to promote protein folding and buffering of misfolded proteins (see poster). When folding fails, chaperones can reroute misfolded proteins to the UPS by recruiting E3 ligases to the misfolded proteins (Hipp et al., 2019). In case the UPS is overwhelmed or proteins escape the chaperone surveillance system, they become targets for the autophagy machinery, which mediates their lysosomal degradation. Notably, autophagy is able to target proteins for degradation even when they cannot be unfolded and thus has a broader substrate spectrum compared to the UPS (see poster) (Dikic, 2017).

Autophagy is in turn divided into three pathways – macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Although all these pathways converge at the lysosome, they are mechanistically distinct. In microautophagy, substrates are delivered into the endolysosomal system through direct invagination of the limiting membrane (Yamamoto et al., 2023). During CMA, substrate proteins are recognized by chaperones, unfolded and delivered into the lysosomal lumen by a channel composed of LAMP2A (an isoform of LAMP2) (Kaushik and Cuervo, 2018). By contrast, in macroautophagy (hereafter called autophagy), the substrates referred to as cargoes are enclosed in *de novo* formed double-membrane vesicles, the autophagosomes (Adriaenssens et al., 2022). Autophagy can target specific cellular cargoes with high specificity, and the pathway targeting aggregated proteins is termed aggrephagy, the subject of this Cell Science at a Glance article. We describe how aggrephagy is mechanistically regulated in physiology and pathology, with a focus on neurons and neurodegeneration.

Different types of protein aggregates

Protein aggregates come in a wide variety of structures and have various potential impacts on cells (see poster). The simplest aggregates are composed of few units of a misfolded protein, often termed oligomers, which are fuzzy structures enriched in exposed hydrophobic patches (Knowles et al., 2014). These properties make them highly reactive species, which is the reason why they might be considered as the most perilous aggregates (Lasagna-Reeves et al., 2012). Oligomers are frequently metastable and continue their (mis)folding trajectory toward higher-order structures, composed of hundreds of protomers with more compact structures (Cremades et al., 2012). Here, the hydrophobic residues are more packed, and the resulting folds can be extremely stable, potentially lasting for decades in living systems (Braak et al., 2011). The path from oligomers to fibrils can proceed through many intermediates, characterized by an increasing amount of compaction (Knowles et al., 2014). This compaction can be also referred to as rigidity. Many rigid protein aggregates adopt the amyloid structure, which is enriched in β-sheet folds (Knowles et al., 2014). The contribution of these higher-order aggregates to pathology is still under debate.

Aggrephagy can intercept these misfolding trajectories at many stages, and mechanistically mapping which aggrephagy pathway(s) degrade a specific kind of aggregate is a core question of the field.

Aggrephagy pathways

The main aggrephagy cascade in eukaryotes starts with the orchestrated action of the cargo receptors p62 (also known as SQSTM1) and the sequestosome-like receptor (SLR) NBR1 (see poster). The main selective trigger for the pathway is ubiquitylation of the cargo proteins (Bjorkoy et al., 2005; Kirkin et al., 2009; Sun et al., 2018; Zaffagnini et al., 2018). Of note, ubiquitin-independent pathways relying on p62 have also been described (Gal et al., 2009; Watanabe and Tanaka, 2011; Xu et al., 2019), some of them triggered by N-terminally arginylated proteins (Cha-Molstad et al., 2015, 2017). Another type of ubiquitin-independent pathway has been reported, which is centered on the removal of aggregates via another SLR, optineurin (Korac et al., 2013).

Both p62 and NBR1 are equipped with UBA domains that recognize ubiquitin, as well as PB1 domains mediating homo- and hetero-oligomerization. In p62, the PB1 domain allows it to oligomerize and to bind to the PB1 domain of NBR1 (Lamark et al., 2003). In contrast to p62, NBR1 is unable to oligomerize via its PB1 domain because it lacks one of the oligomerization interfaces (Ciuffa et al., 2015; Lamark et al., 2003). The p62–NBR1 hetero-oligomer, likely composed of many p62 molecules capped by NBR1, is able to sequester ubiquitylated proteins within larger condensates (Sánchez-Martín et al., 2020; Sun et al., 2018; Turco et al., 2021; Zaffagnini et al., 2018).

Downstream of p62–NBR1-dependent condensate formation, several other factors facilitate autophagy. One of the first factors to be recruited to the condensates is TAX1BP1 via its interaction with NBR1 (Ohnstad et al., 2020; Sarraf et al., 2020; Turco et al., 2021). TAX1BP1 in turn promotes the induction of autophagosome biogenesis at the cargo by the recruitment via its SKICH domain of the core autophagy protein and ULK1 complex subunit FIP200 (also known as RB1CC1) (Ohnstad et al., 2020; Turco et al., 2021). As it grows around the cargo, the nascent autophagosomal membrane is decorated with LC3 proteins, which are bound by the LC3-interacting region (LIR) motifs present in the cargo receptors p62, NBR1 and TAX1BP1 (Adriaenssens et al., 2022). This final step allows the tethering of the cargo to the growing membrane. After the process of autophagosome biogenesis is completed, the cargo is engulfed by a closed double-membrane and directed to the lysosome. Thus, the cargo receptors have a central role in aggrephagy, as they are involved in condensing the cargo, recruiting the machinery for induction of autophagosome biogenesis and tethering the cargo to the membrane.

Regulatory principles and modulators of aggrephagy

The process of aggrephagy must be regulated and fine-tuned in order to avoid the targeting of ubiquitylated proteins that fulfill various functions in the cell or that are destined for degradation by the proteasome.

The first layer of specificity is derived from the biochemical properties of the cargo receptors. The p62 UBA domain has a low affinity for ubiquitin (Long et al., 2010), thus requiring its oligomerization to efficiently capture a cargo. Conversely, the UBA domain of NBR1 has higher affinity for ubiquitin (Walinda et al., 2014), but it is likely underrepresented in condensates due to its lower expression levels (Cho et al., 2022). These multivalent interactions and different binding affinities toward ubiquitin are fundamental to achieve efficient and selective sequestration of cargoes, without targeting all ubiquitylated proteins in the cell. In particular, p62 has a preference for cargoes on which ubiquitin is clustered (Wurzer et al., 2015). Moreover, the UBA domain of p62 is autoinhibited by dimerization (Long et al., 2010) and therefore has a low on-rate with respect to ubiquitin binding.

The second layer of aggrephagy regulation is provided by phosphorylation. For example, the kinases ULK1, part of the ULK1 complex which also includes FIP200, and TBK1, including its scaffold proteins NAP1 and SINTBAD (also known as AZI2 and TBKBP1, respectively), modulate the interaction of several cargo

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receptors with ubiquitin. In addition, TBK1 phosphorylates p62 at serine 403, to increase its affinity for ubiquitin, as well as FIP200 and other components of the aggrephagy cascade (Fu et al., 2018; Lim et al., 2015; Matsumoto et al., 2011; Pilli et al., 2012; Sánchez-Martín and Komatsu, 2018; Schlütermann et al., 2021). Phosphorylation of p62 by ULK1 has been shown to retain KEAP1 within p62 condensates (Ikeda et al., 2022 preprint). KEAP1 negatively regulates the transcription factor NRF2 (also known as NFE2L2), thus integrating aggrephagy with the redox stress response (Ichimura et al., 2013).

A third layer of regulation is derived from the ubiquitin chains on the cargo, with some studies suggesting a preference of cargo receptors for K63 chains (Kirkin et al., 2009; Tan et al., 2008). This implies that the cellular ubiquitylation and de-ubiquitylation machineries are deeply integrated with aggrephagy. Interestingly, very little is known on what ubiquitin 'writers and erasers' modulate the ubiquitylation landscape of protein aggregates.

Apart from these regulatory principles based on post-transcriptional modifications, an ever-increasing number of proteins have been shown to modulate aggrephagy (see poster). A well-described modulator is ALFY (also known as WDFY3), a gigantic protein that serves as a scaffold for aggrephagy, potentially linking p62 to autophagosomal membranes enriched in phosphatidylinositol 3-phosphate (Clausen et al., 2010; Filimonenko et al., 2010). Other examples include WDR81 (Liu et al., 2017), a prefoldin-like protein UXT that enhances p62 clustering in motoneurons (Yoon et al., 2021), and TECPR1, which tethers puromycin-induced aggregates to the autophagosome via LC3 binding (Wetzel et al., 2020). Taken together, many layers of regulation act upon aggrephagy to select which protein aggregates are destined for autophagic degradation.

Chaperone-assisted selective autophagy and other alternative aggrephagy pathways

Chaperone-assisted selective autophagy (CASA) is responsible for the removal of aggresomes, structures containing misfolded proteins forming upon proteasome inhibition (Kawaguchi et al., 2003; Tedesco et al., 2023) (see poster). CASA could be regarded as a specific form of aggrephagy, in which the HSP70 family and HSPB8 chaperones, in coordination with the ubiquitin E3 ligase CHIP (also known as STUB1), recognize misfolded proteins and tag them with ubiquitin (Arndt et al., 2010). Crucial to this pathway is the action of BAG3, a modular protein that connects all factors involved and directs the protein complex to the microtubule-organizing center (Klimek et al., 2017). Aggregates are initially stored in vimentin cages and are eventually targeted to the lysosome for clearance in a piecemeal manner (Kawaguchi et al., 2003).

An alternative pathway involving the TRiC subunit CCT2 was recently discovered, targeting end-stage, rigid aggregates to the lysosome (Ma et al., 2022) (see poster). In this pathway, CCT2 tethers a protein aggregate directly to LC3, facilitating its degradation. Tethering is independent of ubiquitylation, p62, NBR1 and TAX1BP1. Surprisingly, it is also independent from the rest of the TRiC complex. Canonically, TRiC folds a wide array of substrates including cytoskeletal proteins (Gestaut et al., 2022).

Cells might also relieve some of the aggregation burden by secreting protein aggregates. This exocytic secretion can be boosted pharmacologically, for instance by inhibiting PIKFYVE, a kinase involving in vesicle fusion (Hung et al., 2023). Inhibiting PIKFYVE ameliorates pathologies in model systems for amyotrophic lateral sclerosis (ALS), another devastating disease associated with protein aggregation (Hung et al., 2023). Cells have thus evolved a plethora of different pathways to remove protein aggregates. A major task now is to unravel how these pathways are coordinated and how they function in different cell types, including postmitotic cells, such as neurons and muscle cells.

Aggrephagy in the central nervous system and its derailment

Seminal experiments have demonstrated a role for autophagy in guarding the neuronal proteome, as evident by the accumulation of protein aggregates and the development of neurodegenerative phenotypes when autophagy is impaired (Hara et al., 2006; Komatsu et al., 2006). Under physiological conditions, most of the catabolic lysosomal activity of the neuron takes place in the soma (see poster) (Stavoe and Holzbaur, 2019). Away from the soma, autophagy is also active in the synapses (Lieberman and Sulzer, 2020). Systems have thus evolved to transport mature autophagosomes there from distal projections (Cason and Holzbaur, 2022). The condensation effect of cargo receptors might be key for initial collection of misfolded proteins that are located far away from the degradative zones of the neurons under basal conditions. In this light, a protective role of p62 has been described to counteract aggregation (Nezis et al., 2008; Ono et al., 2022). Similarly, a protective role has also been reported for ALFY in delaying the onset of Huntington's disease (Fox et al., 2020). Although we are starting to appreciate how aggrephagy is safeguarding the proteome of neurons, further research is needed to understand how cargo condensation, induction of autophagosome biogenesis and lysosomal degradation are temporally and spatially coordinated.

Under pathological conditions, neurons undergo severe changes in their autophagy flux, with an overall impaired degradative capacity (see poster). Accumulation of autophagic vesicles is, for instance, a signature of AD (Nixon et al., 2005). Autophagy is also abnormally upregulated in axonal spheroids, pathological structures found in AD-affected brains that are also classified as dystrophic neurites (Yuan et al., 2022). The disruption of aggrephagy is evident from the very beginning of the aggrephagy cascade; indeed, p62 accumulates and colocalizes with Tau and α-synuclein amyloid aggregates, respectively, in AD and PD (Kuusisto et al., 2002, 2003).

CASA is also affected in some pathological conditions, with mutations in its components resulting in several protein aggregation diseases, including spinocerebellar ataxia (Tedesco et al., 2023). In AD-affected brains, protein levels of CASA components are increased compared to those seen in unaffected control brains (Koopman and Rudiger, 2020). Furthermore, studies on AD brains showed higher *BAG3* mRNA levels in inhibitory neurons than excitatory ones, the latter being more vulnerable to neurodegeneration, highlighting BAG3 as a potential central node in maintaining neuronal proteostasis (Fu et al., 2019).

Despite the fact that additional aggregate-specific autophagy pathways driven by UXT1 (also known as TRADD) and CCT2 have been described (Ma et al., 2022; Yoon et al., 2021), protein aggregation eventually prevails in various diseases. A possible explanation is that, at some stage, the aggrephagy cascade cannot recruit the downstream components required for autophagosome biogenesis. Alternatively, and not mutually exclusively, the aggregation load might be too high and exceed the degradative capacity of the lysosomal system. Taken together, aggrephagy components have a fundamental role in keeping the neuronal proteome balanced and are dysregulated during neurodegeneration.

Outlook

Many outstanding questions remain unanswered in the aggrephagy field. It is unclear how exactly aggrephagy is integrated into the proteostasis network. In addition, we do not understand how the

different steps of the aggrephagy cascade are spatially arranged in complex cell types, such as neurons, and whether these differ between the various types of neuronal cells. Understanding aggrephagy in other cell types is equally relevant. It is likely that in tissue-specific pathways there are receptors and regulatory mechanisms yet to be discovered. An example of a non-neuronal protein aggregation disease is alcohol-induced liver cirrhosis, which is characterized by the accumulation of p62-positive protein aggregates in hepatocytes (Zatloukal et al., 2002). Beyond individual cells, there is increasing evidence for non-cell autonomous processes for aggregate clearance (Peng et al., 2020; Xie et al., 2022).

Finally, although the reprograming of aggrephagy as a therapeutic strategy to treat diseases caused by accumulation of proteins aggregates holds great promise, they are not yet available in the clinic. However, proof-of-principle for the reprograming of the autophagy pathway for aggregate clearance already exist (see poster). For instance, small molecules that mediate the recruitment of an E3 ligase catalyzing the attachment of K63-linked ubiquitin chains to a target protein for the subsequent recruitment of p62 have been developed. This strategy has been named autophagy-targeting chimera, or AUTAC (Takahashi et al., 2019). In a further study, removal of pathological Tau aggregates was achieved by chemically tethering them to the p62 zinc finger domain (termed AUTOTAC, for autophagy-targeting chimera) (Ji et al., 2022). In addition, small bifunctional molecules (autophagosome-tethering compounds, ATTECs) have been developed to tether Htt aggregates to LC3, routing them to the lysosome (Li et al., 2019). Whereas both AUTACs and AUTOTACs indirectly or directly exploit the activities of cargo receptors, which includes their interactions with the core autophagy machinery, the ATTEC approach bypasses their activities and relies on preformed LC3-positive autophagic membranes. Some of these approaches are now in clinical trials (Garber, 2022), and it will be exciting to see how the modulation of aggrephagy and autophagy in general will be exploited to treat disease and to promote healthy aging (Aman et al., 2021).

Competing interests

Sascha Martens is a member of the scientific advisory board of Casma Therapeutics. All other authors declare no competing or financial interests.

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High-resolution poster and poster panels

A high-resolution version of the poster and individual poster panels are available for downloading at https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.260888#supplementary-data.

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