PROTEIN QUALITY CONTROL

Protein homeostasis from the outside in

Secretory proteins undergo multiple rounds of co- and post-translational quality control checks inside the cell, but how their integrity is maintained outside the cell is an emerging topic. A study establishes a model system to investigate how the extracellular proteome is protected and integrates its findings into existing immune pathways.

Brant M. Webster, Holly K. Gildea and Andrew Dillin

Maintaining the integrity of the proteome is essential for cell viability, yet proteins continuously face destabilising pressures that threaten function[1]. Proteins are therefore subject to constant surveillance by protein homeostasis networks that monitor quality throughout the protein life cycle—from synthesis to folding, trafficking, and degradation[2]. Strikingly, a large portion of the proteome is physically separated from these protective measures, specifically secreted proteins and extracellular domains of integral plasma membrane proteins. Similarly to intracellular proteins, secretory proteins are closely monitored during synthesis and folding in the endoplasmic reticulum[3] (ER) and undergo further quality checks at the Golgi[4] and cytosolic side of the plasma membrane[5]. The existence of these spatially segregated, yet overlapping quality control measures prompts the question "What happens if a protein misfolds after it is secreted?" In the same vein as "If a tree falls in a forest and no one is around to hear it, does it make a sound?," if a protein misfolds outside the cell, does it know? Does the cell, tissue, or organism care?

A spectrum of diseases featuring deposits of extracellular aggregates emphasises the dangers of misfolded extracellular proteins if left unchecked[6]. A growing consensus of data propose a general strategy of extracellular protein homeostasis: holdases bind to and prevent the aggregation of misfolded proteins while directing them for endocytosis and lysosomal degradation[6]. Yet, the mechanisms of extracellular protein homeostasis remain poorly defined and are hindered by a lack of genetically tractable model organisms with physiologically relevant extracellular compartments. In a recent *Nature* study, Gallotta and colleagues reconciled these compounding challenges by leveraging the genetic power of *Caenorhabditis elegans* to define the genetic requirements of extracellular protein homeostasis[7] (Fig. 1).

In previous work, the authors defined the *C. elegans* aggregation-prone proteome and discovered a number of proteins, like LBP-2 and LYS-7, known to be secreted into the pseudocoelom, the worm's extracellular space[8]. Here, Gallotta et al. characterise the nature of secretory protein aggregation by tracking the fate of fluorophore-tagged secretory proteins. They confirm that detergent-insoluble LBP-2 aggregates accumulate in the pseudocoelom, not in the intracellular compartments. Notably, they were not able to detect considerable LBP-2 aggregation in young animals, but observed an increasing prevalence of extracellular LBP-2 aggregates with age, consistent with an age-dependent collapse described for other intracellular quality control networks[9].

In the worm, extracellular proteins are endocytosed and removed from the pseudocoelom by a group of cells called coelomocytes[10]. The authors demonstrate that ablation of coelomocyte cells increases the number of aggregates in the pseudocoelom and decreases lifespan, suggesting that endocytosis plays a key role in removing aggregation-prone extracellular proteins and promotes the integrity of the extracellular proteome. However, it remains unclear whether these misfolded and aggregated proteins are preferentially endocytosed over soluble forms and whether endocytosis is mediated directly by coelomocyte receptors.

To identify extracellular factors that promote the integrity or clearance of aggregation-prone proteins in the extracellular space, the authors performed an RNA interference screen targeting transcripts that encode soluble secreted proteins. Gallotta and colleagues asked which genes, when knocked down, aggravate LBP-2 aggregation. They identified 57 genes that when knocked down increased LBP-2 aggregation in the extracellular space, which the authors coined extracellular regulators, or ECRs. The authors focus on a group of 13 ECRs and demonstrate that they physically engage aggregating proteins in the extracellular space. They also show that overexpression of individual ECRs can reduce the aggregation of LBP-2 and LYS-7. Notably, the authors demonstrate that overexpression of a subset of ECRs can even extend lifespan, which is the first set of direct evidence implicating extracellular proteome homeostasis in longevity.

Though the functions of these ECRs remain predominantly uncharacterised, the authors tested whether the expression of the ECRs change with age. Consistent with their findings of increasing aggregation with age, they observed that expression of several ECR genes decreases with age. This loss of expression led the authors to speculate that transcriptional programs may regulate the expression and activity of ECRs. Indeed, there was a strong correlation with ECR genes and transcripts upregulated by pathogen attack.

Worms have a primitive innate immune system that consists of a mitogen-activated protein kinase (MAPK) signalling pathway that activates expression of gene products that protect against pathogenic attack[11]. The authors determined that activation of the immune response using pathogenic bacteria or exposure to virulence factors induces expression of several ECRs through canonical MAPK-dependent signalling. Notably, the authors demonstrate that this activation results in decreased accumulation of LBP-2 extracellular aggregates. Surprisingly, while overexpression of ECRs is not sufficient to activate immune response pathways on its own, expression of specific ECRs does promote resistance to pathogenic attack and highlights a functional relationship between extracellular quality control and the immune response.

In light of growing appreciation for the role immune responses play in pathophysiology of neurodegenerative diseases[12], we are particularly intrigued by the interaction between extracellular quality control and the immune response in the worm. The authors speculate that this interaction may simply be a manifestation of the requirement of a healthy extracellular proteome to fight pathogens. It may also be suggestive of a more elaborate interdependency. For example, the ER

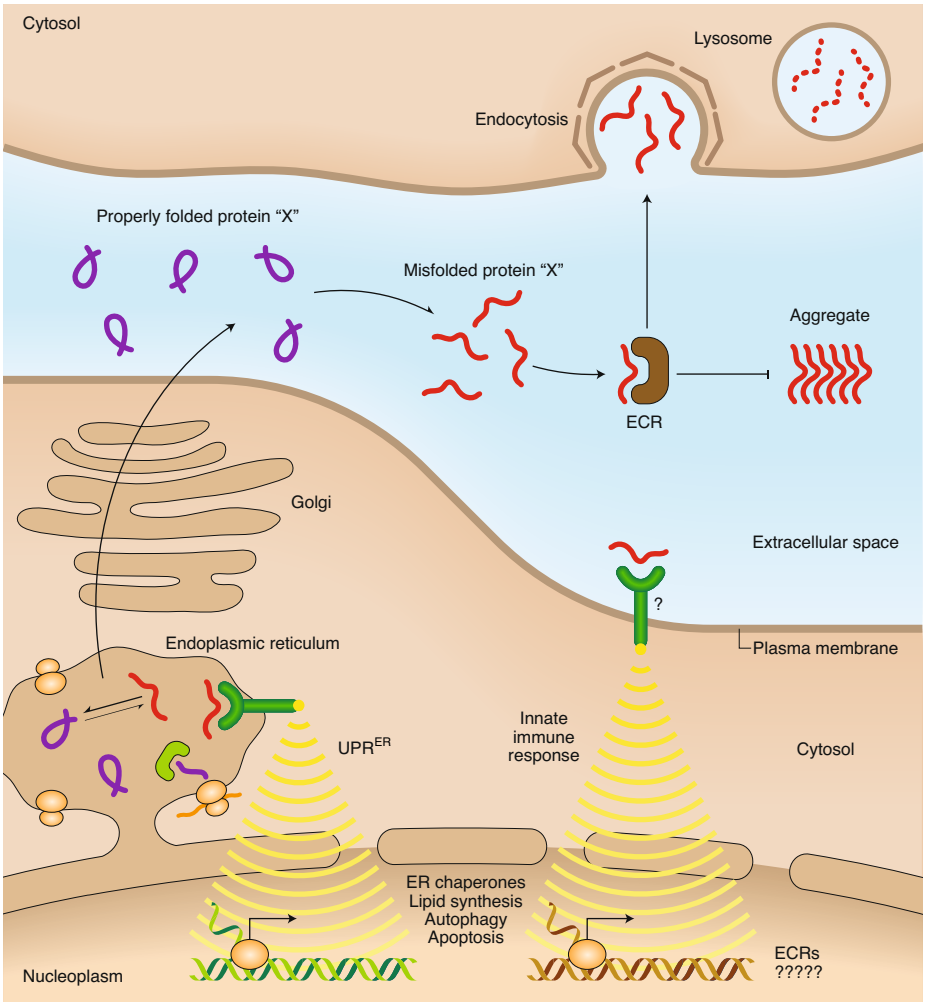

Fig. 1 | Overview of secretory protein homeostasis. Ribosomes synthesise secretory proteins into the endoplasmic reticulum (ER) lumen. Chaperones (green) promote proper folding of nascent proteins and refolding of damaged secretory proteins (orange). Build-up of damaged proteins in the ER activates a signalling cascade called the ER unfolded protein response (UPR^{ER}) that upregulates the expression of specific gene products to restore proper folding balance and ensure the quality and secretion of properly folded proteins (purple). Outside the cell, extracellular regulators (ECRs, brown) bind to misfolded proteins, preventing aggregation and promoting clearance by endocytosis followed by lysosomal degradation. Data presented here suggest the existence of an ER-like surveillance of extracellular protein quality that conveys extracellular damage to the nucleus via immune pathways to increase expression of ECR gene products.

unfolded protein response (UPR^{ER}) surveils the quality of newly synthesised secretory proteins to balance protein-folding needs. Surpassing ER protein-folding capacity activates signalling pathways that relieve stress and mitigate damage[13]. Recent work suggests that the UPR^{ER} also plays critical roles in protecting cells from misfolded, aggregation-prone proteins outside the cell[14]. In the way that the UPR^{ER} protects the extracellular space from the 'inside-out', we wonder whether immune-response pathways sense misfolded extracellular proteins and activate extracellular UPR-like activities to protect cells and tissues from the 'outside in'.

Together this work establishes a model organism and functional framework for future studies using *C. elegans* to investigate protein quality control outside the cell. Importantly, the emerging set of rules governing the integrity of the worm extracellular proteome seem to be in line with mammalian models. This work introduces a facet of extracellular protein homeostasis comprising a putative UPR-like signalling cascade to protect against the toxic effects of misfolded proteins outside the cell with broad basic and clinical implications. We predict that the genetic amenability and short lifespan of the worm combined with tracking of endogenous aggregation-prone proteins in intact organisms holds immense potential, allowing for comprehensive understanding of the role of extracellular quality control in tissue and organismal physiology. ❐

Brant M. Webster[1], Holly K. Gildea[2] and Andrew Dillin[1,2,3]✉

[1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA. [2]Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, CA, USA. [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA, USA.

✉*e-mail: dillin@berkeley.edu*

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Competing interests

The authors declare no competing interests.