Cell Biology Select

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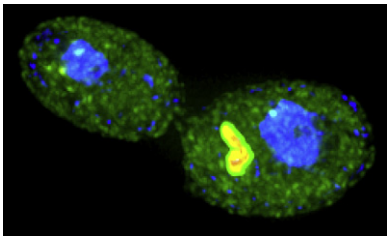
The accumulation of misfolded proteins occurs during cellular stress and aging and is a common feature of many neurodegenerative disorders. Recent work provides insights into the mechanisms by which cells detect and respond to the presence of misfolded proteins. This includes the discovery that misfolded proteins preferentially accumulate in one of two cellular compartments. Other new findings reveal proteins involved in the retrotranslocation of misfolded glycoproteins, uncover mechanisms regulating proteolysis of misfolded proteins, and suggest new strategies for the treatment of diseases associated with protein aggregation.

Misfolded Proteins Have a Parting of Ways



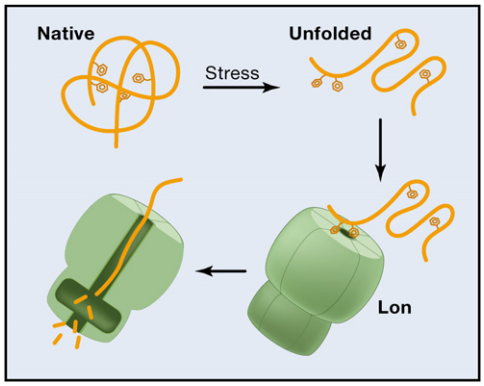
A prion inclusion in yeast surrounded with Hsp104-GFP. Image courtesy of J. Frydman.

According to new work by Kaganovich et al. (2008), different classes of misfolded proteins partition to two separate intracellular compartments, one next to the nucleus (juxtanuclear) and the other near vacuoles (perivacuolar). By examining the cellular distribution of multiple misfolded proteins in both yeast and mammalian cells, the authors propose a stunningly simple model: soluble ubiquitinated proteins go to the juxtanuclear compartment, whereas insoluble terminally misfolded proteins accumulate in the perivacuolar compartment. In addition to the spatial segregation, the fate of the proteins in these two compartments appears to be equally divergent. For example, proteins in the juxtanuclear compartment are in close proximity to cytoplasmic concentrations of the 26S proteasome, whereas the perivacuolar compartment is marked by proteins implicated in autophagy. Although in most cases the juxtanuclear compartment appears to be the compartment of first resort for protein quality control, the authors demonstrate that disease-causing Huntingtin and prion proteins preferentially partition to the perivacuolar compartment. Is this preferential sorting a mechanism of cellular protection that sequesters these dangerous proteins for long-term storage and disposal? Or does this reflect the fact that these misfolded proteins interact inefficiently with the normal quality-control machinery and as a consequence are shunted to the perivacuolar pathways by default? Although this question is unresolved, the answer may be highly relevant to understanding the pathogenesis of diseases associated with protein misfolding. Intriguingly, the authors demonstrate that enhancing the ubiquitination of a prion protein enhances its partitioning to the juxtanuclear compartment. Likewise, blocking the ubiquitination of proteins that normally go to the juxtanuclear compartment leads them to partition to the perivacuolar protein. If enhancing the partitioning of misfolded proteins that cause disease to one of these compartments were to lessen their toxicity, these efforts might form the basis of a cellular assay with which researchers can screen for new therapeutic compounds.

*D. Kaganovich et al. (2008). Nature **454**, 1088–1095.*

Lon Takes in the Aromatic Fragrance of Unfolded Proteins

How do you clear toxic aggregates of unhealthy proteins that are irreversibly misfolded while leaving healthy proteins intact even if temporarily misfolded? In their current work, Gur and Sauer (2008) confront this question, studying the mechanisms by which Lon, an AAA^+ protease from the bacterium *Escherichia coli*, recognizes permanently damaged proteins. The authors examined the degradation of a fragment of β-galactosidase (amino acids 3–93), previously shown to be a substrate of Lon, and reveal that a particular 20 amino acid segment of the β-galactosidase fragment facilitates Lon recognition. Moreover, if this 20 amino acid segment is added to other proteins, it enhances their recognition and degradation by Lon. This effect is shown to be due specifically to a patch of aromatic residues. Aromatic amino acids, because of their marked hydrophobicity, are the residues mostly likely to be buried in proteins in their native conformations. Indeed, in native full-length β-galactosidase, the aromatic residues critical for Lon recognition of the β-galactosidase fragment are buried deep within the three-dimensional structure. The authors suggest that there is likely a dynamic interplay between proteases, such as Lon, that degrade misfolded proteins and chaperones that assist refolding. They note that the sequences that Lon recognizes in its substrates occur less frequently than do the sequences recognized by the chaperones DnaK and DnaJ. From this, they propose that the relative abundance of recognition sites for proteases and chaperones could provide a mechanism by which permanently damaged proteins are eliminated, while transiently misfolded proteins are spared proteolytic degradation.



The protease Lon recognizes hydrophobic residues in misfolded proteins. Figure courtesy of E. Gur.

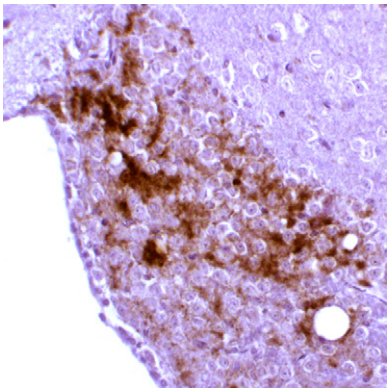
*E. Gur and R. T. Sauer (2008). Genes Dev. **22**, 2267–2277.*

Unruly Glycoproteins Are Discharged from the ER by SEL1L and Partners

Misfolded secreted and membrane proteins are discharged from the endoplasmic reticulum (ER) for proteolytic degradation in the cytosol. Recent efforts by Mueller et al. (2008) identify new proteins in the ER of mammalian cells that mediate the dislocation of misfolded glycoproteins. The authors sought to understand the function of human SEL1L, an adaptor protein in the ER previously implicated in glycoprotein dislocation. They expressed a tagged version of SEL1L in HeLa cells and identified copurifying proteins by mass spectrometry. In addition to previously known interactors, such as the ubiquitin E3-ligase HRD1, the authors report new elements of the dislocation machinery in mammalian cells. Among these are the E2 ubiquitin-conjugating enzyme UBC6e, the ancient ubiquitous protein 1 (AUP1), and the UBX domain containing protein 8 (UBXD8). They also provide evidence from the overexpression of wild-type and dominant-negative constructs that each of these proteins is critical to glycoprotein dislocation. Hence, this work, which more fully characterizes the elements of the mammalian dislocation machinery, is a starting point for determining how the activities of these ER-membrane-bound proteins are coordinated in the removal of misfolded glycoproteins.

B. Mueller et al. (2008). Proc. Natl. Acad. Sci. U.S.A. ***105****, 12325–12330.*

HSF1 Provides Protection from PrP



Aggregates of the scrapie prion (brown) in the caudate nucleus of mice lacking HSF-1. Image courtesy of G. Hutter.

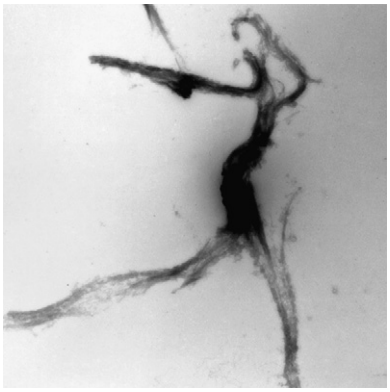
Protein misfolding is a common feature of many forms of neurodegeneration. In a mouse model of prion disease, Steele et al. (2008) now show that the transcriptional regulator heat shock factor 1 (HSF1) extends life span, and does so without affecting the age of symptom onset. The authors examined the survival of wild-type and HSF1 knockout mice after inoculation with murine-adapted scrapie prions. Scrapie leads to neurodegeneration in sheep and goats and is closely related to bovine spongiform encephalopathy ("mad cow disease"). The authors observe that the time to the onset of symptoms, both histological and behavioral, is similar between wild-type and HSF1-deficient mice (~4.5 months after inoculation with the prion). Yet, the progression of the disease is markedly accelerated in HSF1-deficient mice. Given that HSF1 triggers the expression of heat shock proteins, which assist in protein folding, the authors examined whether loss of HSF1 altered the accumulation of prion protein (PrP) aggregates. However, the accumulation of PrP aggregates, as measured by proteinase K digestion, appeared to be similar between wild-type and HSF1-deficient mice. Although HSF1 does not appear to alter the accumulation of PrP aggregates, future work may show whether it alleviates a more generalized form of protein-folding stress brought on by PrP toxicity. Despite uncertainty as to the mechanism of protection, these findings suggest that enhancing the activity of HSF1 might be beneficial in some disease contexts and that a therapeutic strategy targeting HSF1 would not necessarily require intervention at a presymptomatic stage.

A. D. Steele et al. (2008). Proc. Natl. Acad. Sci. U.S.A. ***105****, 13626–13631.*

Aggregates Set Off Factor XII

Activation of Factor XII, a serine protease in blood plasma, mediates multiple processes, including vasodilation, pain, and inflammation. Although Factor XII can be activated in experimental settings by surface materials with a negative charge, such as glass, the physiological regulators of Factor XII remain undiscovered. A new report by Maas et al. (2008) suggests that in vivo, Factor XII is poised to detect the presence of aggregates of misfolded proteins in the blood. Previous work has found that the activity of Factor XII is elevated in the cerebral spinal fluid of patients with Alzheimer's disease. In the new work by Maas et al., the authors provide evidence that the response of Factor XII to misfolded protein may be a general phenomenon. They report that Factor XII activation is elevated in patients with systemic amyloidosis and discover that it is misfolded aggregates and not amyloid fibrils that are key to activating Factor XII. Factor XII then activate kallikreins, which are serine proteases that cleave precursor proteins to liberate inflammatory mediators, such as bradykinin. Interestingly, contact with surfaces also initiates coagulation of blood, but Maas et al. show that the activation of Factor XII in response to misfolded proteins leads only to kallikrein activation, and does not initiate coagulation, suggesting that activation of kallikrein-kinin system can occur independent of the coagulation cascade. Future work may explore the molecular mechanisms by which Factor XII detects and becomes activated by aggregates of misfolded protein.

C. Maas et al. (2008). J. Clin. Invest. ***118****, 3208–3218.*



Aggregates of amyloidosis-related immunoglobulin light chains are an activator of Factor XII. Image courtesy of C. Maas and M. Gebbink.

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