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PROTEIN FOLDING

Different sorting strategies

> “…misfolded proteins sequester differentially in two distinct […] compartments.”

The formation of amyloid inclusions of aggregated proteins, which are typical of many neurodegenerative diseases, has been well documented. But what is the fate of ‘normal’ misfolded cytosolic proteins? And why do some misfolded proteins accumulate in amyloid inclusions, whereas others get degraded?

Judith Frydman and colleagues studied the fate of a fluorescently tagged temperature-sensitive Ubc9 mutant (Ubc9^{ts}) that misfolds above 30°C. In yeast cells, misfolded Ubc9^{ts} transiently accumulated in a juxtanuclear inclusion until it was degraded by the ubiquitin–proteasome pathway. Impairment of proteasome-mediated degradation, however, caused Ubc9^{ts} to accumulate in two distinct inclusions: the juxtanuclear inclusion and a large perivacuolar inclusion.

Another misfolded protein, unassembled von Hippel-Lindau (VHL) tumour suppressor, accumulated just in the juxtanuclear inclusion in yeast cells even in the presence of a proteasome inhibitor. But by raising the temperature to 37°C, VHL accumulated in two distinct inclusions, as observed for Ubc9^{ts}. So, misfolded proteins are sequestered in two distinct intracellular inclusions; the juxtanuclear inclusion forms first and is more prevalent under normal conditions, whereas stress conditions lead to protein accumulation in the second peripheral inclusion.

The sequestration pattern of Ubc9^{ts} and unassembled VHL in mammalian cells was similar to that in yeast. The amyloidogenic mutant huntingtin protein colocalized with the perivacuolar inclusion but not with the juxtanuclear inclusion in yeast and in mammalian cells. So, different types of misfolded proteins sequester differentially in two distinct, evolutionarily conserved, quality-control compartments.

Using fluorescence loss in photobleaching (FLIP) analysis, the authors examined the diffusion properties of fluorescently tagged misfolded proteins in the inclusions. A rapid loss of fluorescence corresponding to Ubc9^{ts} in the juxtanuclear inclusion suggests that misfolded proteins in this ‘juxtanuclear quality control’ compartment (or JUNQ) are soluble and can exchange with the cytosolic pool. By contrast, the perivacuolar compartment retained most of its fluorescent signal, which implies that it contains mostly non-diffusing, insoluble Ubc9^{ts} (hence the name ‘insoluble protein deposit’ or IPOD).

Proteasomes concentrate near JUNQ but not IPOD, which suggests that misfolded protein degradation takes place in JUNQ. The chaperone Hsp104 colocalizes with both JUNQ and IPOD; Hsp104 probably has a refolding purpose in JUNQ, whereas it may fragment protein aggregates in IPOD.

So how are misfolded proteins sorted to JUNQ or IPOD? Impairing ubiquitylation of the misfolded proteins Ubc9^{ts} and VHL blocked their accumulation into JUNQ and instead resulted in their accumulation on IPOD, even at 30°C and in the absence of proteasome inhibition. Blocking their ubiquitylation was associated with reduced solubility; either ubiquitylation status or solubility may determine to which compartment these proteins are sorted. Conversely, polyubiquitylation of prion protein Rnq1 promoted its partitioning to JUNQ.

By sorting Ubc9^{ts} to either JUNQ or IPOD by changing its ubiquitylation state and then reverting to the permissive temperature, the authors showed that polyubiquitylated Ubc9^{ts} in JUNQ can be refolded by Hsp104, whereas non-ubiquitylated Ubc9^{ts} in IPOD is terminally aggregated. So, the accumulation of misfolded proteins together with proteasomes and chaperones in JUNQ may facilitate both degradation and refolding, whereas sequestration of terminally aggregated proteins in IPOD may facilitate clearance, for example, through the autophagic pathway.

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ORIGINAL RESEARCH PAPER Kaganovich, D. *et al.* Misfolded proteins partition between two distinct quality control compartments. *Nature* **454**, 1088–1095 (2008)