Organelle and proteome quality control mechanisms: how cells are able to keep calm and carry on

Jeffrey L. Brodsky[a], Alexey Merz[b], and Tricia Serio[c]
[a]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; [b]Departments of Biochemistry and Physiology and Biophysics, University of Washington, Seattle, WA 98195-3750; [c]Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721

Cells must thrive in a variety of stressful environments. Moreover, a significant number of proteins fold slowly or inefficiently, so there is a constant threat that misfolded proteins might accumulate. An unmitigated response to these stresses can result in cell death. To offset the catastrophic effects of cellular stress, proteins are subject to quality control checkpoints that target aberrant polypeptides for degradation or for refolding via the action of molecular chaperones. In addition, defective or superfluous proteins, lipids, and organelles can be selected for destruction, or are inherited asymmetrically during cell division. Finally, inducible transcriptional programs facilitate protein triage pathways. The "Organelle and Proteome Quality Control Mechanisms" session at the 2013 ASCB Annual Meeting focused on each of these events.

Protein quality control and the cytoplasmic–nuclear nexus

The first talk in this session was delivered by **Thibault Mayor** (University of British Columbia), who highlighted the mechanism that leads to the degradation of ubiquitinated, cytoplasmic proteins in heat-stressed yeast cells. After incubation at elevated temperatures, damaged cytosolic proteins are ubiquitinated and routed to the proteasome. By screening for mutants in which this phenomenon was compromised, Mayor and colleagues identified Hul5 as the ubiquitin ligase that modifies most of these substrates (Fang *et al.*, 2011). Hul5 resides in the nucleus, but, upon heat stress, a significant population migrates to the cytoplasm. Consistent with this model, yeast expressing a form of Hul5 that remains trapped in the nucleus are unable to fully recover from heat shock and exhibit decreased levels of ubiquitinated cytoplasmic proteins. Current efforts are defining how heat-damaged proteins are selected for ubiquitination, assessing whether Hul5 acts as a bona fide E3 ligase or as a ubiquitin extension enzyme (i.e., an E4), and identifying other ligases that function with Hul5.

Chris Guerriero from the Brodsky Laboratory (University of Pittsburgh) described new chimeric proteins that can be used to investigate specific questions underlying the selection of substrates for endoplasmic reticulum–associated degradation (ERAD) and cytoplasmic quality control. Each chimera contains a single "degron," which derives from a well-characterized yeast ERAD substrate, Ste6p*. When expressed in the cytosol, the degron undergoes Hsp70- and proteasome-dependent degradation (Guerriero *et al.*, 2013). Examining substrate ubiquitination in a new in vitro assay provided evidence to suggest that Hsp70 acts both before and after substrate ubiquitination. Surprisingly, degron ubiquitination and degradation utilized a nuclear resident E3 ligase, San1. This result is in contrast to the E3 requirements for the degradation of a degron-containing protein tethered to the ER. In this case, proteolysis via the ERAD pathway is mediated by different E3 ligases, even though degradation remains Hsp70 dependent.

A presentation by **Shengyun Fang** (University of Maryland School of Medicine) provided another example of how misfolded proteins are selected and routed to different compartments for degradation. Fang and colleagues discovered that the chemical entrapment of a mammalian nuclear export factor, Crm1, led to the aggregation of ERAD substrates in the nucleus. Some substrates also accumulated in this compartment when p62, an autophagy delivery factor, was silenced. Therefore, a shuttle escorts misfolded proteins from the nucleus to the cytosol and, in some cases, to the autophagic machinery. Ongoing work is exploring the substrate selectivity of Crm1 and p62, and identifying how these factors facilitate the delivery of ubiquitinated and nonubiquitinated proteins from the nucleus. Combined with the results from Mayor and Guerriero, these data emphasize how cellular compartments communicate to mediate protein quality control.

Modulating substrate interactions

Kevin Morano (University of Texas Health Science Center at Houston Medical School) addressed Hsp70 regulation. Hsp70s interact dynamically with substrates through an ATPase cycle (Zuiderweg *et al.*, 2013). Three families of nucleotide exchange factors (NEFs) regulate Hsp70 nucleotide release: Hsp110, HspBP1, and Bag. The yeast NEFs were shown to maintain protein homeostasis, with Hsp110 promoting protein folding on its own and degradation of a misfolded substrate in coordination with HspBP1. Reflecting these roles, loss of HspBP1 alone or in combination with Hsp110 induces a stress response and chaperone elevation, presumably due to the accumulation of misfolded proteins. Yet, the NEFs were dispensable for recovery after heat shock, suggesting that nucleotide exchange may not be a limiting event once Hsp70 levels are elevated.

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Address correspondence to: Jeffrey L. Brodsky (jbrodsky@pitt.edu).

Tricia Serio (University of Arizona) next discussed how the presence of one chaperone substrate promotes the refolding of another. The yeast prion Sup35 assembles into amyloid aggregates in vivo, which are amplified through fragmentation by the AAA+ATPase Hsp104 (Tuite and Serio, 2010). While the amyloid is normally stable, it is efficiently lost at elevated temperature (Newnam *et al.*, 2011). This loss depends not only on the elevation of chaperone expression but also on the formation of heat-induced aggregates of other proteins. It was shown that Hsp104 engagement with heat-induced aggregates increases its levels in a subset of cells, which promotes amyloid disassembly. Thus, substrate interactions can promote chaperone accumulation beyond expression-level changes.

Organelle quality control

The final presentations described emerging work on quality control at the whole-organelle level. Damaged mitochondria contribute to aging and neurodegeneration, and proteins that target damaged mitochondria have emerged (PINK1, Parkin, Nix, Atg 32). Nevertheless, the mechanisms of selectivity are incompletely understood. **Hagai Abeliovich** (Hebrew University) described a dramatic autophagic turnover of mitochondria (mitophagy) when yeast cells are cultured under stationary-phase conditions. Turnover requires both mitochondrial fission and fusion. Proteomic analyses revealed subpopulations of mitochondrial proteins degraded at divergent rates, again dependent on fission and fusion. Abeliovich *et al*. (2013) speculate that protein subsets are retained or turned over through phase transition–like condensations (Brangwynne, 2013).

Alex Merz (University of Washington) described links between the SNARE disassembly proteins Sec17 and Sec18 (NSF and α-SNAP) and SM proteins essential for SNARE-mediated fusion. SM function was needed to withstand Sec17 and Sec18 overproduction, and SMs directly protected SNARE complexes from disassembly. Unexpectedly, Sec17 stimulated formation of 1:3:1 SNARE–Sec17–SM cocomplexes. Merz *et al*. propose that SMs stimulate fusion by augmenting SNARE function and protecting incipient complexes from premature disassembly. The system taken as a whole has properties consistent with a kinetic proofreading system that accelerates on-pathway membrane traffic while eliminating complexes that lead to incorrect fusions or irreversible organelle aggregation.

REFERENCES

Abeliovich H, Zarei M, Rigbolt KT, Youle RJ, Dengjel J (2013). Involvement of mitochondrial dynamics in the segregation of mitochondrial matrix proteins during stationary phase mitophagy. Nat Commun 4, 2789.

Brangwynne CP (2013). Phase transitions and size scaling of membrane-less organelles. J Cell Biol 203, 875–881.

Fang NN, Ng AH, Measday V, Mayor T (2011). Hul5 HECT ubiquitin ligase plays a major role in the ubiquitylation and turnover of cytosolic misfolded proteins. Nat Cell Biol 13, 1344–1352.

Guerriero CJ, Weiberth KF, Brodsky JL (2013). Hsp70 targets a cytoplasmic quality control substrate to the San1p ubiquitin ligase. J Biol Chem 288, 18506–18520.

Newnam GP, Birchmore JL, Chernoff YO (2011). Destabilization and recovery of a yeast prion after mild heat stress. J Mol Biol 408, 432–448.

Tuite MF, Serio TR (2010). The prion hypothesis: from biological anomaly to basic regulatory mechanism. Nat Rev Mol Cell Biol 11, 823–833.

Zuiderweg ERP, Bertelsen EB, Rousaki A, Mayer MP, Gestwicki JE, Ahmad A (2013). Asllostery in the Hsp70 chaperone proteins. Top Curr Chem 328, 99–154.