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Protein Quality Control and Elimination of Misfolded Proteins of the Endoplasmic Reticulum and the Cytosol: Pathways and Mechanisms

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Proper folding of all cellular proteins is essential for life. However, this process is far from being error-free. Misfolding of proteins can originate from mutations, unbalanced protein subunit synthesis or mistakes that accompany every cellular process, preventing a protein to find its native conformation. In addition, damaging conditions like oxidation even lead to unfolding of properly folded proteins. However, aberrant proteins are extremely harmful to the cell. Therefore cells must have a potent system to recognize misfolded proteins as well as a highly efficient elimination machinery. In mammalian cells a defective recognition and degradation machinery results in severe diseases as Alzheimer, Parkinson, Creutzfeldt-Jakob disease in humans or bovine spongiform encephalopathy (BSE) in cattle, to name some of the most prominent of the many diseases linked to a defective removal of protein waste (1). On the other hand in some cases "hyper" activity of the system eliminates mutant but otherwise biologically active protein as is the case for a mutated cystic fibrosis transmembrane conductance regulator (CFTR) leading to cystic fibrosis in humans (1). Using yeast as a eukaryotic model organism we study the recognition and elimination machineries of misfolded proteins, involved in an event, which is considered to be a "housekeeping" process of all eukaryotic cells. We elucidate this housekeeping process in two major protein folding compartments, the endoplasmic reticulum (ER) and the cytosol.

We have shown in yeast that degradation of CPY*, a soluble mutated and by this misfolded carboxypeptidase yscY protein of the vacuole (lysosome) is recognized in the ER as being misfolded and retrograde transported back out of this cellular compartment into the cytoplasm where it is degraded via the ubiquitin-proteasome system (2, 3). N-glycosylation is crucial for recognition of this misfolded CPY* protein, part of the recognition components being α-1,2-mannosidase and Yos9p (4-6). In contrast to membrane bound derivatives of CPY*, degradation of soluble CPY* requires the ER-lumenal Hsp70 chaperone Kar2p (BiP) (7). With the help of several ER-membrane proteins (Der1p, Der3/Hrd1p, Hrd3p) CPY* reaches the cytosol, where it is polyubiquitinated (8-10). Delivery of the polyubiquitinated misfolded protein to the proteasome for degradation requires the trimeric Cdc48-Ufd1-Npl4 complex as well as the UBA-UBL proteins Dsk2p and Rad23p of the cytosol for their degradation (11-13). We further show that ER import defective CPY* (ΔssCPY*), derivatives thereof (e.g., ΔssCPY*-GFP) or ER import incompetent wild type CPY (ΔssCPY), remaining in the cytosol, are also rapidly degraded via the cytosolic ubiquitin proteasome system. In contrast to the soluble ERAD substrate CPY*, degradation of these proteins is independent of the trimeric Cdc48 complex as well as of Dsk2p and Rad23p. Instead, their degradation requires the cytosolic Hsp70 chaperones of the Ssa type as well as the co-chaperone Ydj1p. The Hsp90 machinery is not involved in the degradation process. Elimination of malfolded ΔssCPY*-GFP is dependent on the ubiquitin conjugating enzymes Ubc4p and Ubc5p. While proteasomal degradation of signal sequence deleted, cytoplasmically localized ΔssCPY*-GFP requires the Ssa1-machinery, degradation of a GFP fusion containing the C-terminal 37 amino acid region of ornithine decarboxylase, a ubiquitin-independent degradation tag (GFPcODC) is independent of this machinery. Most interestingly, fusing Δ ssCPY* to GFPcODC to form ΔssCPY*-GFPcODC reimposes a dependence on the Ssa1 chaperone for degradation. Obviously, the malfolded protein domain dictates the route of protein elimination. We propose that Ssa1p fulfills several functions in the degradation process of misfolded proteins: remodel and solubilize precipitated material, keeping it soluble and deliver it to the proteasome (14).