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Published in final edited form as:
Nature Cell Biology. 2014 Dec 1 ; 16(12): 1130-1132 | doi: 10.1038/ncb3079
Nature Publishing Group ►

Rsp5/Nedd4 clears cells from heat-damaged proteins.

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Protein quality control systems protect cells from proteotoxicity caused by the accumulation of aberrantly folded polypeptides. The Rsp5/Nedd4 ubiquitin ligase is now identified as a major constituent of a clearance pathway that degrades misfolded cytosolic proteins after exposure to heat.

Errors during folding and exposure to harmful conditions may obstruct the structure of proteins and give rise to polypeptides that are trapped in an aberrant conformation. Such malfolded species fail to fulfill their designated function and are prone to aggregation. Protein misfolding threatens health because it causes proteotoxic stress that is associated with conformational pathologies like Huntington's and Alzheimer's disease[1]. The accumulation of defective polypeptides is sensed by cellular protein quality control systems (PQCs). In eukaryotic PQCs molecular chaperones act in concert with ubiquitin (ub-) ligases to sequester terminally misfolded polypeptides from intermediates of productive folding and route them for proteasomal degradation[2,3]. Therefore, the ability to discriminate folded from structurally defective forms of a protein is an intrinsically important property of PQCs. The activity of ub-ligases appears to make a fundamental contribution to this process. Several of these enzymes are involved in cytoplasmic PQCs, but it is poorly understood how they target their substrates. For example, molecular chaperones act as substrate recognition factors of the mammalian ub-ligase CHIP, which facilitates ubiquitylation and degradation of the selected misfolded proteins[4]. By contrast, the yeast ub-ligase San1 directly binds hydrophobic patches exposed on the surface of aberrantly folded proteins. The selection relies on small disordered domains in San1[5]. This ligase may also functionally team up with molecular chaperones and another ub-ligase, Ubr1, for the elimination of malfolded proteins[6,7]. Recent work identified the proteasome-associated ligase Hul5 as being involved in protein ubiquitylation following heat-shock[8]. How this enzyme targets its substrates is thus far unclear.

A manuscript in this issue of *Nature Cell Biology* introduces another cytoplasmic PQC ub-ligase[9]. Thibault Mayor and co-workers noticed, that heat-shocked cells pile up much less ubiquitylated material when the yeast ub-ligase Rsp5, or its mammalian counterpart Nedd4, is impaired. This observation indicates that Rsp5/Nedd4 is involved in the disposal of cytoplasmic proteins damaged by heat. The authors recently reported on Hul5 as an ub-ligase involved in cytoplasmic heat-shock PQC[8] and therefore continued to investigate the functional relationship between the enzymes. Surprisingly, each ligase seems to target distinct sets of substrates in heat-shocked cells. This implies the development of diverging cellular strategies for the processing of the large heterogeneity of defective proteins that may arise under stress conditions[9]. Moreover, the two ligases employ different types of poly-ub chains to label their targets: Hul5 substrates appear to be predominantly decorated with lysine 63-linked chains, whereas Rsp5 clients seem to be modified with lysine 48-linked poly-ub. This fits the author's

observation that bulk protein turnover following heat-shock requires proteasomal activity. However, *in vitro* studies demonstrate that Rsp5 catalyzes mono- or lysine 63-linked poly-ub on substrates and most of the reported functions of this enzyme have been connected with this activity[10,11]. By assuming the involvement of additional ub-modifying enzymes this potential discrepancy can be solved. Rsp5 may attach single ub moieties on selected client molecules, which serve as primers for the elongation by other ub-ligases (Figure 1). Indeed, polyubiquitylation of RNA-polymerase II occurs by such a two-step mechanism with Rsp5 initiating ubiquitylation with mono-ub, which are then modified with lysine 48-linked poly-ub by the Elc1/Cul3 complex[12].

Rsp5/Nedd4 is an ub-ligase of the HECT-domain family involved in highly diverse cellular activities like transcription factor activation, regulation of RNA polymerase II and ribosome degradation. Most importantly, Rsp5 is also involved in receptor endocytosis and endocytic sorting. The abundance of membrane proteins at the cell surface is regulated by endocytosis in a signal- or conformational dependent manner. From the endocytic compartment, membrane proteins are either recycled or subjected to proteolysis in lysosomes (or the yeast vacuole)[11]. Mayor and colleagues now provide the exiting insight that the Rsp5 dependent cytoplasmic PQC pathway shares principles of substrate recognition with endocytosis/endosomal sorting. Rsp5 substrates of this pathway typically contain a short stretch of amino acid termed the PY motif, that confers binding to WW-domains in the ligase and thereby provokes their ubiquitylation[11]. Strikingly, the authors discover that a large portion of the Rsp5 heat-shock substrates also harbors sequences reflecting PY or PY-like motifs[9]. Such a PY motif, for example, is located near the dimerization domain of Cdc19. Rsp5 binds to this protein for ubiquitylation in cells exposed to high temperature. Pyk2, which shares a high However, Mayor and colleagues find that insertion of a PY stretch into the corresponding region of Pyk2 facilitates Rsp5-dependent ubiquitylation of Pyk2 following heat exposure. The authors also note that the PY motifs in Rsp5 heat shock substrates are preferentially located in regions that are predicted to be highly structured. It is tempting to speculate that these motifs are usually buried in tightly folded domains and become exposed only when the structural integrity of the proteins is affected (Figure 2). Remarkably, a variant of Cdc19 harboring a mutation that weakens dimer formation is ubiquitylated by Rsp5 even at only modestly elevated temperatures. This finding strongly implies that at least some cytoplasmic proteins contain internal quality control signals like the PY motif that mediate binding to specific ub-ligases.

Still, many of the Rsp5 heat shock substrates do not contain obvious PY stretches. Mayor and colleagues demonstrate that Rsp5 can recognize damaged proteins by an alternative mode[9]. Rsp5 is known to team up with several adaptor proteins that contain PY motifs. These co-factors recruit cargo lacking the PY consensus and thereby regulate the activity of Rsp5 towards appointed substrates[11]. In heat-treated cells, they see that Rsp5 rapidly associates with Ydj1, an Hsp40-type co-chaperone that contains a PY motif[9]. Further, deletion of *YDJ1* or expression of a Ydj1 variant lacking the PY motif impairs the accumulation of ubiquitylated proteins in heat-shocked cells. This observation suggests that Ydj1 assists in the selection of proteins that lose their shape at elevated temperatures, thereby expanding the range of Rsp5 clients. Indeed, variants of Cdc19 that lack the PY stretch are still targeted by Rsp5, however, in contrast to the wild-type protein, Ydj1 is now required for this process.

The data presented by Mayor and colleagues suggest a model in which similar recognition modes account for Rsp5 function in endocytosis of plasma membrane proteins and for its function in elimination of heat damaged proteins. Conformational changes in Fur4, a high affinity uracil importer, triggers ubiquitylation by Rsp5 and transport to lysosomes[13]. The amino terminal part of Fur4 encompasses sequence elements sensing the conformational status of the protein. Strikingly, this domain also contains a putative PY motif raising the possibility that Rsp5 directly binds to Fur4 in response to structural switches. A topologically related methionine transporter Mup1, which lacks such a PY-like stretch, also undergoes Rsp5-dependent internalization. Yet unlike Fur4, the efficient down-regulation of Mup1 relies on Rsp5 adaptor proteins of the Art family[13,14].

Internal quality control signals represent an efficient way to integrate ub-ligases into various PQC pathways (Figure 2). The so-called N-end rule pathway, for example, has recently been shown to remove orphan subunits of protein complexes once their N-acetylated amino termini are not protected by associated partner proteins[15]. It is also obvious that a convenient positioning of such quality control signals within a protein offers the opportunity to detect already small aberrations in a protein structure. Mild denaturing conditions are supposed to cause minor defects rather than overall obstructions in the folding of a number of proteins. Such aberrant species are likely to escape detection by PQC ub-ligases like San1 designed to ubiquitylate heavily misfolded proteins. Therefore, this ligase appears not to play a major role in heat-shock-induced ubiquitylation[8]. Rsp5 in contrast seems to be adopted to recognize even slightly heat-denatured proteins. However, following association with the co-factor Ydj1 it may recognize proteins displaying more global features of misfolding.

What remains to be unraveled is the mode of ubiquitylation distinguishing the two pathways. Rsp5 mounts mono- or short lysine 63-linked ub-chains on substrates. This is sufficient to drive endocytosis. But which enzyme elongates these chains with lysine 48-linked ub-chains in the case of heat denaturation? What is the signal that distinguishes both classes of substrates so that one is further modified while the other remains as it is? Could it be simply a difference in localization of the two classes of substrates? Further experimentation is needed to answer these questions.

References

1. Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M. & Ulrich Hartl, F. *Annu. Rev. Biochem.* **82,** 323–355 (2013).
2. McClellan, A. J., Tam, S., Kaganovich, D. & Frydman, J. *Nat. Cell Biol.* **7,** 736–741 (2005).
3. Buchberger, A., Bukau, B. & Sommer, T. *Mol. Cell* **40,** 238–252 (2010).
4. McDonough, H. & Patterson, C. *Cell Stress Chaperones* **8,** 303–308 (2003).
5. Rosenbaum, J. C. *et al. Mol. Cell* **41,** 93–106 (2011).
6. Prasad, R., Kawaguchi, S. & Ng, D. T. W. *Mol. Biol. Cell* **21,** 2117–2127 (2010).
7. Guerriero, C. J., Weiberth, K. F. & Brodsky, J. L.. *J. Biol. Chem.* **288,** 18506–18520 (2013).
8. Fang, N. N., Ng, A. H. M., Measday, V. & Mayor, T. *Nat. Cell Biol.* **13,** 1344–1352 (2011).
9. Fang, N. N. *et al. Nat. Cell Biol.* **16,** 1227–1237 (2014).
10. Kim, H. C. & Huibregtse, J. M. *Mol. Cell. Biol.* **29,** 3307–3318 (2009).

11. Rotin, D. & Kumar, S. *Nat. Rev. Mol. Cell Biol.* **10,** 398–409 (2009).
12. Harreman, M. *et al. Proc. Natl. Acad. Sci.* **106,** 20705–20710 (2009).
13. Keener, J. M. & Babst, M. *Traffic* **14,** 412–427 (2013).
14. Zhao, Y., MacGurn, J. A., Liu, M. & Emr, S. *eLife* **2,** (2013).
15. Shemorry, A., Hwang, C.-S. & Varshavsky, A. *Mol. Cell* **50,** 540–551 (2013).

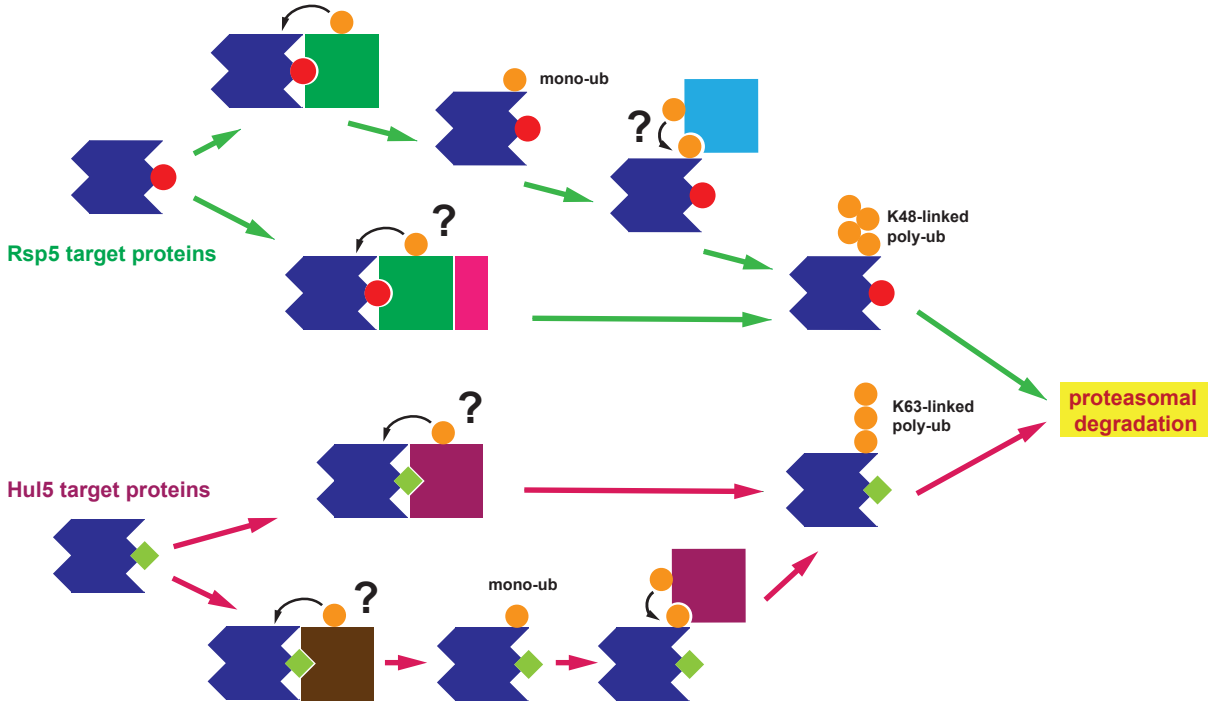

Figure 1. Proposed activity of Rsp5 and Hul5 in the clearance of heat-damaged proteins. Structurally compromised proteins (dark blue) expose distinct features (red circle and light green diamond, respectively) that route them to Rsp5 or Hul5 ubiquitylation pathways. Rsp5 (green) may directly bind to exposed PY motifs or target its clients via adaptor proteins (not shown). Most likely Rsp5 attaches mono-ubiquitin (ub) to substrates, which primes them for modification by another ligase with lysine 48-linked (K48) poly-ub. Alternatively, specific co-factors (pink) may shift the activity of Rsp5 towards direct formation of lysine 48 poly-ub chains. It is not clear, whether Hul5 (purple) can directly access structurally aberrant proteins. Eventually, this ligase processes substrates that are first targeted by other PQC enzymes (brown) and catalyzes the formation of lysine 63-linked (K63) poly-ub chains on them. K48- and K63-poly-ub serve as signals for proteasomal degradation.

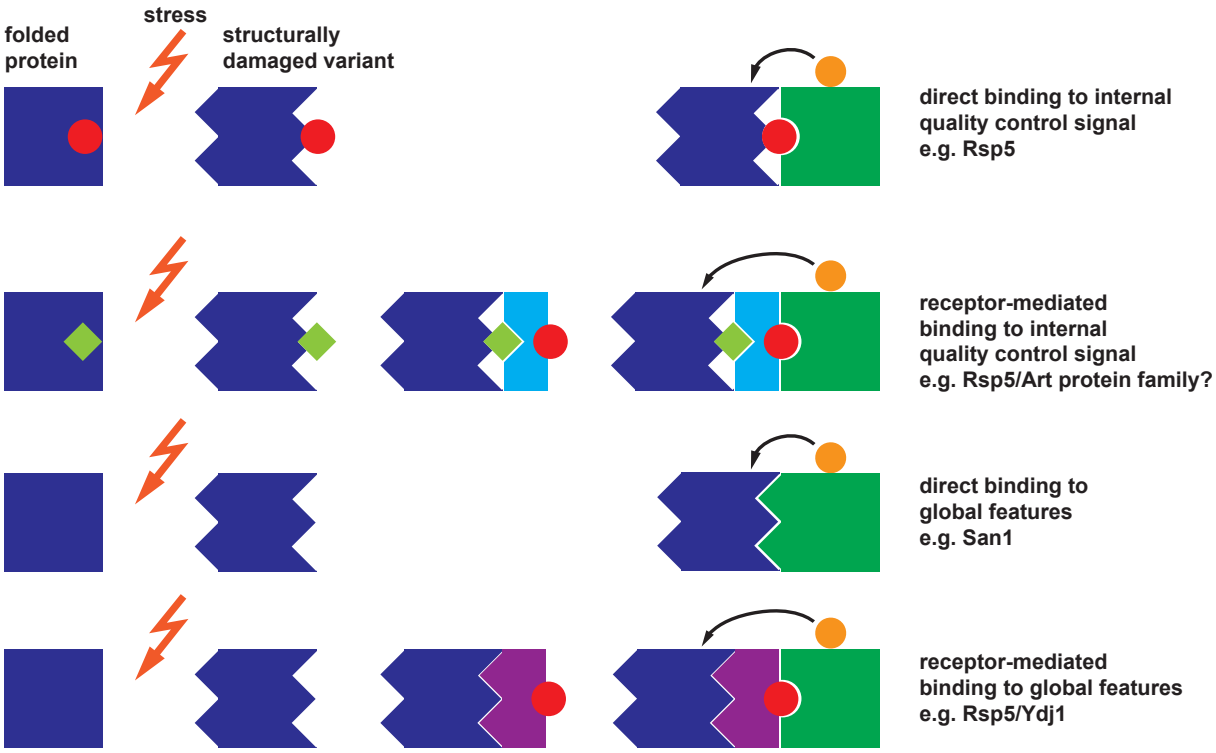

Figure 2. Distinct ways to integrate ubiquitin ligases into protein quality control pathways.
Folding stress (orange arrow) like heat-shock damages proteins (dark blue). Such aberrant polypeptides eventually expose internal quality control signals like the PY motif (red circle) that are concealed within the structure of the productively folded form. Direct binding of ub-ligases (dark green) to these signals triggers the transfer of ubiquitin (orange circle) to the target. Alternatively, adaptor proteins (light blue) may recruit proteins exposing specific internal quality control signals (light green diamond) to the ub-ligases. Other ub-ligases contain sequence elements that allow the interaction with client proteins displaying characteristic attributes of global structural obstructions like surface-exposed hydrophobic patches. Appointed adaptors (purple) like Ydj1 may also target such structurally compromised proteins to ub-ligases designed to bind specific quality control signals.