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ER Dislocation: Cdc48p/p97 Gets Into Dispatch the AAAct

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Misfolded or unassembled proteins present in the lumen of the endoplasmic reticulum are exported to the cytosol and degraded. Recent studies have implicated a complex containing the AAA ATPase Cdc48p/p97 in the export process.

Newly synthesised proteins in the endoplasmic reticulum (ER) of eukaryotic cells are subjected to a stringent quality control system [1]. They are only retained or transported to their final destination if they fold and, where appropriate, assemble correctly. Those that fail to do so are exported to the cytosol, and if glycosylated they are then deglycosylated. Degradation occurs within the proteasome, usually following ubiquitination. According to current models, export of such failed proteins to the cytosol uses the same protein-conducting channel, the Sec61 translocon, that mediates the import of newly made proteins into the ER [2]. Certain viruses have evolved to co-opt this 'ER-associated degradation' (ERAD) pathway for their own advantage. For example, the human cytomegalovirus proteins US2 and US11 down-regulate the immune response by dislocating the major histocompatibility complex (MHC) class I heavy chain from the ER membrane [3]. The dislocated MHC class I heavy chain molecules are apparently recognized as ERAD substrates, resulting in their export and degradation, and thus preventing their normal defensive role in presenting virally-derived peptides to the immune system at the surface of the infected cell.

The mechanisms by which cells recognize misfolded proteins in the ER are beginning to emerge. The glycans of *N*-glycosylated proteins, for example, have been implicated in the selection of degradation substrates in a process that may be mediated by ER-located lectins, and candidate proteins that might be responsible for this recognition/targeting have been recently identified [4,5]. The folding state of proteins within the ER is constantly monitored, and the protein maintained in a folding-competent conformation, by interaction with a range of ER molecular chaperones and, where appropriate, enzymes that catalyse post-translational modifications such as disulphide bond formation.

While such interactions define whether a protein is correctly folded and likely biologically active, it is less clear how the actual export step is achieved for misfolded proteins that fail this structural surveillance. Two recent studies [6,7] have begun to shed light on this question by demonstrating that Cdc48p/p97 — one of the 'ATPases associated with various cellular activities' or 'AAA' ATPases — and its partners are required to transport proteins from the ER to the cytosol.

Previous studies have led to the conclusion that Cdc48p/p97 is able to participate in distinct cellular processes, such as membrane fusion and ubiquitin-dependent protein degradation, by interacting with different adaptors specific for a particular process. Yeast Cdc48p and its mammalian cell homologue p97 mediate certain homotypic organellar membrane fusion events. Cdc48p is required for the fusion of ER membranes [8], and the gene encoding it was first identified in a cell-cycle mutant that arrests in mitosis with an undivided nucleus. p97 is involved in Golgi membrane fusion during cisternal reassembly from mitotic Golgi fragments [9]. Both proteins form a homohexameric ring structure. During the fusion of mitotic Golgi fragments, p47 links p97 to its substrate, the t-SNARE syntaxin 5 [10], while a different complex of p97 bound to Ufd1p and Npl4p was postulated for p97 to function in ubiquitin-dependent processes [11].

In eukaryotes, proteasome-mediated degradation is primarily achieved after the covalent conjugation of ubiquitin to the target protein. Both yeast and mammalian Cdc48p/p97 proteins have been shown to be required for the degradation of certain ubiquitin-containing substrates [12,13]. Consistent with this role in protein degradation, Cdc48p has been shown to bind to the several proteins implicated in the ubiquitin fusion degradation (UFD) pathway [14], such as Ufd3p [12], while mammalian p97 binds both a ubiquitinated degradation substrate and the proteasome itself [15]. Recently, yeast Cdc48p, Ufd1p and Npl4p have been implicated in the proteasome-mediated processing of ER-bound proteins [16]. Furthermore, members of the AAA ATPase family are directly involved in the degradation of integral membrane proteins in both bacteria and mitochondria, where they actively extract transmembrane segments and transport solvent-exposed protein domains across a membrane [17,18].

The above considerations, coupled to the fact that a significant proportion of both Cdc48p and p97 is associated with the ER membrane, prompted Ye *et al.* [6] to examine their potential involvement in ERAD. Firstly they determined the stability of misfolded ER proteins in *cdc48* strains of *Saccharomyces cerevisiae.* They tested an ER membrane protein, the mouse MHC class I heavy chain H-2K^{b}, and the misfolded lumenal protein CPY*, an aberrant form of yeast vacuolar carboxypeptidase Y (CPY), both of which had previously been shown to be rapidly degraded ERAD substrates in yeast. Both proteins were found to be significantly stabilized in *cdc48* mutant cells relative to wild-type cells. Transport of native CPY from the ER, via the Golgi, to the vacuole occurred as normal in the mutant cells. The results also confirmed the

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involvement of the two adaptor proteins Ufd1p and Npl4p in the degradation of ER proteins in yeast. Both H-2K^b and CPY* were significantly stabilised in Ufd1p- or Npl4p-defective strains. When *cdc48*, *ufd1* or *npl4* mutants expressing stabilised CPY* were fractionated, CPY* was recovered in a membrane fraction where it was resistant to added protease. This suggested that a complex of Cdc48p, Ufd1p and Npl4p might function in the export of misfolded protein substrates across the ER membrane.

Bays *et al.* [7] independently identified *NPL4* as a yeast gene required for the degradation of hydroxymethyl glutaryl-CoA reductase, a membrane-bound ERAD target protein. Substrate ubiquitination was not impaired in a strain bearing a temperature-sensitive mutation in the *NPL4* gene, placing the role of Npl4p at a post-ubiquitination, but pre-proteasomal, step in the ubiquitin–proteasome pathway. Again, each member of the putative Cdc48p–Ufd1p–Npl4p complex was found to be individually required for ERAD.

Several lines of evidence presented by Ye *et al.* [6] confirmed that p97 has a similar role in mammalian cells to that of Cdc48p in yeast. The experimental system used human cytomegalovirus US11 protein-induced retrotranslocation and proteasomal degradation of MHC class I heavy chains, and a *trans* dominant negative mutant of rat p97 in which two point mutations reduced its capacity to hydrolyse ATP at least ten-fold. Astrocytoma cells expressing US11 were permeabilized and either wild-type or mutant p97 was added at about a three-fold excess over endogenous protein. Collectively, the data obtained showed that p97, most likely acting in concert with its partners, is required for the export of MHC class I heavy chains from the ER to the cytosol. Intriguingly, the addition of mutant p97 inhibited the release of ubiquitinated chains to the cytosol, but did not affect the total amount of ubiquitinated heavy chains which accumulated on the membrane and were sensitive to protease treatment.

A Cdc48p–Ufd1p–Npl4p complex is therefore likely to be involved in ERAD in both yeast and mammals. They may act by directly extracting proteins from the ER membrane in an ATP-dependent manner, analogous to the way that FtsH in *Escherichia coli* and AAA proteases in mitochondria appear to 'pull' proteins from the respective membranes for degradation [17,18], or by assisting the membrane translocation step itself (Figure 1).

The mechanism by which the Cdc48p–Ufd1p–Npl4p complex mediates the dislocation of ubiquitinated ERAD substrates likely involves the proposed chaperone function of the complex, which appears to be specifically directed to ubiquinated substrates. Indeed, the yeast Cdc48p–Ufd1p–Npl4p complex has been recently shown to be required for the selective extraction of a ubiquitinated protein from a protein complex present on the cytosolic face of the ER [19], in a process that has been proposed to rely on the ubiquitin-binding activity of Cdc48p/p97 [13,19]. Further biochemical characterization of the Cdc48p–Ufd1p–Npl4p complex will be needed to determine whether the chaperone activity is actually required during the passage through the ER membrane, or to release the ubiquitinated protein from the ER cytosolic surface. Nor is the system likely to be confined to yeast and mammals: the *Arabidopsis thaliana* genome contains Cdc48p/p97 homologues, at least one of which can functionally complement a yeast *CDC48* mutant [20].

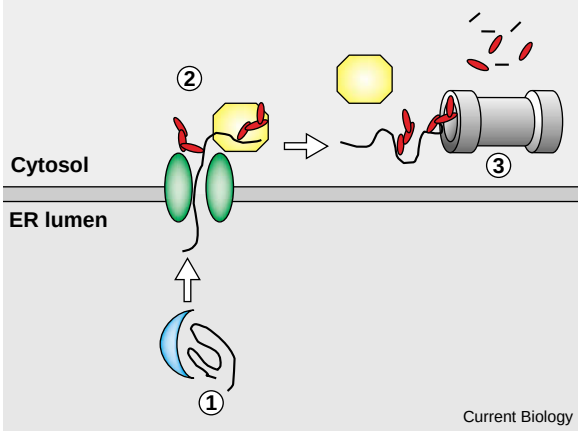

Figure 1. A model for the role of Cdc48p in ERAD.

ERAD substrates are maintained in a retrotranslocation competent state by binding to ER-located chaperones (blue), and targeted for retrotranslocation (**1**). The Cdc48p–Ufd1p–Npl4p complex (yellow) may act directly on (ubiquitinated) ERAD substrates engaged in a translocation channel (green) (**2**). Once released from the ER membrane, the ERAD substrate is presented to the proteasome (grey) for degradation (**3**). All events can be tightly coupled. Polyubiquitin chains (red) are added to the ERAD substrate when it appears on the cytosolic face of the ER membrane, and may be involved in initial recognition by the Cdc48 complex. Dislocation is dependent on the ATPase activity of the Cdc48 protein.

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