

EGAD! There is an ERAD doppelganger in the Golgi.

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Disposal of membrane proteins in the late secretory pathway is thought to be exclusively facilitated by ESCRT-dependent lysosomal degradation. In this issue of *The EMBO Journal*, Schmidt and colleagues define a previously uncharacterized endosome and Golgi associated degradation (EGAD) pathway. This pathway, which has remarkable similarities to ERAD in the endoplasmic reticulum, operates in post-ER organelles via the proteasome, contributes to lipid homeostasis in eukaryotic cells.

Membrane proteins define a large and diverse class of proteins in eukaryotic cells. At the plasma membrane and at the surface of cellular organelles membrane proteins play a myriad of functions, from nutrient uptake and sensing to protein and lipid synthesis and trafficking.

With such important and widespread functions, the activity and abundance of membrane proteins must be tightly regulated. Scrutiny on membrane proteins starts during their biogenesis in the endoplasmic reticulum (ER). To ensure that only folded and functional membrane proteins traffic to other cellular membranes, the ER is equipped with a stringent quality control process called ER-associated degradation (ERAD) (Ruggiano *et al*, 2014). All protein molecules that fail to properly fold become ERAD substrates. Upon recognition, substrates are ubiquitinated, translocated to the cytoplasm and membrane extracted by the AAA+ ATPase Cdc48 before their release in the cytoplasm for degradation by the proteasome. Central to the execution of these ERAD steps are E3-ubiquitin ligase complexes embedded in the membrane of the ER. In yeast, where ERAD is best characterized, there are three ERAD E3 ligase complexes- the Hrd1, Doa10 and Asi1 complexes- which have different substrate specificities and localize to distinct ER domains (Ruggiano *et al*, 2014; Foresti *et al*, 2014). Together, these ERAD branches prevent the accumulation of misfolded and mislocalized membrane proteins.

ERAD also controls the abundance of some folded proteins. In these cases, the degradation is regulated by metabolic signals, of which the best examples are the enzymes 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR) and squalene monooxygenase (SM), essential for sterol biosynthesis at the ER (Hampton *et al*, 1996; Foresti *et al*, 2013). In sterol-depleted cells both enzymes are relatively long lived however if specific sterol metabolites accumulate in the ER membrane, both HMGR and SM are quickly degraded by Hrd1 and Doa10 ERAD complexes, respectively (Hampton *et al*, 1996; Foresti *et al*, 2013; Ruggiano *et al*, 2014). Regulated degradation of HMGR and SM was shown to be important for sterol homeostasis from yeast to man, underlying important functions of ERAD beyond protein quality control (Ruggiano *et al*, 2014). Whether ERAD also participates in homeostatic regulation of other lipids such as glycerophospholipids or sphingolipids, whose biosynthesis is at least in part localized to the ER remains unclear.

In post-ER compartments, disposal of membrane proteins was thought to depend only on lysosomal degradation, through a process involving the endosomal sorting complex required for transport

(ESCRT)- complex (Migliano & Teis, 2018). Ubiquitinated membrane proteins at surface of endosomes/multivesicular bodies (MVBs) are recognized by ESCRT machinery which promote their sorting and internalization into intra-luminal vesicles, a process that also requires the AAA-ATPase Vps4. Subsequent MVB fusion with the lysosome, results in the release of intraluminal vesicles in the hydrolytic lumen of the lysosome and ultimately in the degradation of their content (Migliano & Teis, 2018).

In this issue of EMBO J., Schmidt and colleagues set out to identify additional mechanisms of protein degradation operating in post-ER compartments (Schmidt *et al*, 2019). They hypothesized that components of such a system would become essential in the absence of a functional ESCRT pathway such as in cells lacking the AAA+ ATPase Vps4. Using a genetic approach, they identified Tul1, an E3-ubiquitin ligase and subunit of the Dsc (Defective in SREBP cleavage) complex. Other Dsc complex subunits also became essential in cells lacking ESCRTs suggesting a critical function of the whole Dsc complex under these conditions.

In *S. pombe*, where it was originally discovered, the Dsc complex contains six components: the membrane proteins Tul1, Dsc2, Dsc3, Dsc4, Ubx3 as well as the cytosolic AAA+ ATPase Cdc48 (Stewart *et al*, 2011). Interestingly, the membrane components of the Dsc complex share structural homology with the ERAD Hrd1 E3 ligase complex. In fission yeast, the Dsc complex was mostly characterized for its role in the activation of the transcription factor Sre1, homologous to the mammalian sterol regulatory element-binding protein (SREBP). SREBPs are transcription factors synthesized as an inactive precursor bound to the ER membrane. Under conditions of low sterols (in mammals) or oxygen (in fission yeast), SREBP cleavage-activating protein (SCAP) promotes the traffic of SREBPs from ER to the Golgi for cleavage, resulting in the release of the active SREBP transcription factor (Stewart *et al*, 2011). Sre1 activation in the Golgi requires ubiquitination by the Dsc E3 ligase complex, followed by proteolytic cleavage by the rhomboid Rbd2 assisted by Cdc48 (Stewart *et al*, 2011; Hwang *et al*, 2016). However, in the absence of Rbd2, the Dsc complex targets the immature SREBP precursor for proteasomal degradation (Hwang *et al*, 2016). This observation is at odds with work in budding yeast, where Tul1 substrates appeared to be degraded exclusively via the lysosome (Migliano & Teis, 2018). Moreover, a recent study described two mutually exclusive Dsc co-factors, Vld1 and Gld1, defining Dsc complexes with distinct subcellular distribution (Yang *et al*, 2018). While Gld1-containing Dsc complex localizes to Golgi/endosomes, Vld1-containing complex localizes to the membrane of the vacuole, the yeast equivalent to the lysosome (Yang *et al*, 2018).

To identify Dsc complex substrates whose turnover is independent of lysosomes, Schmidt and colleagues resorted to quantitative proteomics. Following up on one substrate, Orm2, they showed that Tul1 and all other Dsc subunits were necessary for Orm2 degradation. Interestingly, Vld1 was dispensable and Orm2 degradation depended exclusively on Gld1. Consistently, deletion of Tul1 or other Dsc subunits resulted in Orm2 accumulation at the Golgi/endosomes. Orm2 degradation also required Tul1-dependent ubiquitination, Cdc48-mediated membrane extraction and proteasome activity, a sequence of events highly reminiscent of ERAD but in this case operating in a post ER-compartment, at the level of Golgi/endosomes. This mode of degradation of membrane proteins by the Dsc complex was coined Endosome/Golgi associated degradation (EGAD) (Schmidt *et al*, 2019).

Orm2 is an integral membrane protein predominantly localized to the ER where it negatively regulates sphingolipid biosynthesis in yeast, a function that it shares with its paralog Orm1. Orm1 and 2 bind and inhibit the serine:palmitoyl-coenzyme A transferase (SPT), the first enzyme in sphingolipid biosynthesis (Breslow *et al*, 2010). In response to low sphingolipid levels, Orm1 and 2 are inactivated via TORC2-Ypk1 mediated phosphorylation, relieving SPT inhibition and allowing sphingolipid synthesis (Roelants *et al*, 2011). Phosphorylated Orm2 is exported from the ER and, as it reaches

Golgi/endosomes, is degraded by EGAD. Indeed, Orm2 phospho-mutants cannot be detected outside of the ER and, as a consequence, are largely resistant to EGAD degradation and display lower sphingolipid precursors such as ceramides. A comparable sphingolipid defect is also observed in Orm2 ubiquitination-resistant mutants and *tu1Δ* cells indicating that both Orm2 phosphorylation and EGAD-mediated degradation contribute to regulate SPT activity. This role of EGAD in controlling sphingolipid levels through regulated Orm2 degradation has similarities with the mechanism by which ERAD maintains sterol homeostasis.

Interestingly, Orm1 is not an EGAD substrate and appears to be regulated only through phosphorylation. Whether this is because phosphorylated Orm1 is not efficiently exported from the ER or it is not recognized by the Dsc complex is unclear. Also, how Orm2 phosphorylation triggers its export is unknown. A simple possibility is that phosphorylated Orm2 has reduced affinity for SPT facilitating its trafficking out of the ER. Importantly, it is intriguing that Orm2 needs to traffic to Golgi/endosomes for degradation, as regulated degradation of other proteins such as HMGR and SM occurs in the ER. Given that the final stages of sphingolipid synthesis occur in the Golgi, one could speculate that Orm2 turnover in the Golgi may be coupled to an additional regulatory step, such as the binding or sensing of a sphingolipid metabolic intermediate.

Characterization of Orm2 degradation was important in uncovering and outlining the features of EGAD. However, it is unlikely to be its sole (or even main!) substrate. Other potential Dsc substrates were identified and their future characterization will bring new insight into EGAD. Additionally, understanding how the Dsc complex substrates are selected merits further investigation. It will also be important to clarify whether the Dsc complex plays a general role in quality control, for example by degrading substrates that escape ERAD surveillance or undergo misfolding in post-ER compartments. Such a role of EGAD in degradation of misfolded proteins would further strengthen his resemblance to ERAD, as if it were its identical twin!

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