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SPOTLIGHT

Chaperoning transmembrane helices in the lipid bilayer

Qi Zhang and Yihong Ye

Elimination of membrane proteins often requires recognition of their transmembrane domains (TMDs) in the lipid bilayer. In this issue, Arines et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202001116) show that in *Saccharomyces cerevisiae*, the vacuole-associated Rsp5 ubiquitin ligase uses a TMD in substrate adaptor Ssh4 to recognize membrane helices in Ypq1, which targets this lysine transporter for lysosomal degradation during lysine starvation.

In eukaryotic cells, protein quality control (PQC) mediates the degradation of not only aberrant but also unwanted polypeptides, safeguarding both the quality and quantity of the cellular proteome (1). A central goal in PQC research is to delineate the mechanism of substrate selection, which, if inappropriately executed, could lead to undesired destruction of functional proteins and thus the collapse of the proteostasis network. For soluble proteins that succumb to PQC, it is usually the surface exposure of hydrophobic elements that alerts cellular chaperones to potential folding catastrophe (2). Chaperones often serve a dual triaging role: while giving their clients additional time to fold, they can also interface with degradation machineries such as the ubiquitin proteasome system or lysosomes, causing the elimination of terminally misfolded or unwanted polypeptides.

Unlike PQC of soluble proteins, substrate recognition for membrane proteins bearing abnormal transmembrane domain (TMD) is largely unknown, even for the best characterized PQC process, ER-associated degradation (ERAD; 3). Early studies on PQC of unassembled T cell receptor α chain (TCRα) showed that the single TMD of TCRα contains two charged residues, which are thermodynamically unfavored in the lipid environment and thus must be shielded when TCRα assembles with CD3σ. Accordingly, unassembled TCRα is eliminated by ERAD via a mechanism dependent on these charged residues (4), but TMD-specific chaperones responsible for recognizing charged residues in the lipid bilayer have not been identified. Likewise, recent investigations into the function of the Hrd1 ubiquitin ligase suggested a role for the TMDs of Hrd1 in recognition of specific aberrant membrane proteins in ERAD (5). Cryo-EM studies further showed two juxtaposed central cavities with a lateral gate poised to receive TMDs in the yeast Hrd1 complex (6), but how aberrant TMDs in ERAD substrates are recognized and retrotranslocated by Hrd1 remains an open question.

The issue of substrate recognition becomes even more complex for feedback-regulated degradation of unwanted membrane proteins. In this case, substrates are initially stable and functionally essential, but a change in environmental cues renders them dispensable and results in a short-lived fate. One such example is the sterol-regulated degradation of a sterol-synthesizing enzyme called HMG-CoA reductase (HMGR). HMGR is a stable ER protein when the sterol level is low, but an increase in membrane sterol abundance alters the conformation of a sterol-sensing domain in HMGR, exposing an element functionally equivalent to a degron in short-lived proteasomal substrates (7). Despite extensive studies, the molecular signature of the degron in HMGR is still undefined, let alone the molecular basis of its recognition. In this issue, Arines and colleagues investigate how Ypq1, a multi-spanning lysine transporter of the yeast vacuole, is regulated by lysine availability, a regulated membrane protein turnover event analogous to HMGR degradation. Their study identifies critical residues in Ypq1 TMDs for its turnover and establishes the Rsp5 ubiquitin ligase adaptor Ssh4 as a TMD-specific chaperone that recognizes these elements (8).

Ypq1 is a seven-transmembrane, PQ loop–containing lysine transporter localized to the yeast vacuole membrane. Under lysine-replete conditions, Ypq1 is stable as it uses a PQ loop–dependent conformational cycle to import excess lysine into the vacuole. When lysine is depleted, Ypq1 is sorted into the multivesicular body (MVB) for degradation (Fig. 1). This process is initiated once Ypq1 is ubiquitinated by the ubiquitin ligase complex Rsp5–Ssh4, but how Ypq1 is targeted by Rsp5–Ssh4 has been unclear (9).

To understand the mechanism of Ypq1 recognition, Arines et al. first engineered a Ypq1 mutant that uncouples ligase-mediated

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Correspondence to Yihong Ye: yihongy@mail.nih.gov.

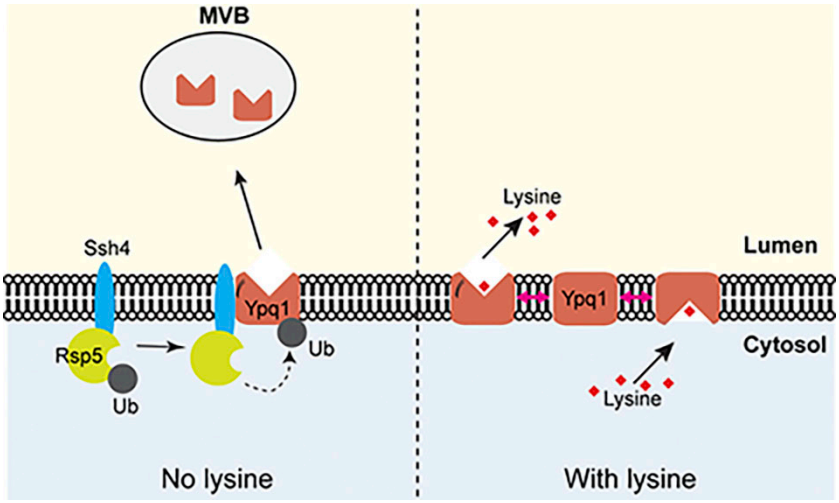

Figure 1. **Regulated recognition of Ypq1 by Ssh4.** When lysine in the cytosol is abundant, Ypq1 undergoes a rapid conformational cycle to transport lysine from the cytosol into the vacuole lumen. Under lysine-depleted conditions, the transporter is trapped in a conformation recognizable by Ssh4, which recruits Rsp5 to catalyze Ypq1 ubiquitination and internalization into the MVB. Ub, ubiquitin.

degradation from lysine availability. This Ypq1 mutant is constitutively degraded in an Ssh4-dependent manner even under lysine-replete conditions. With this tool in hand, they performed a random mutagenesis-based suppressor screen, which identified many suppressor mutants. Mapping these mutations revealed several elements in Ypq1 that are critical for ligase recognition, which include two TMDs (TM5 and TM7) and a cytosolic loop. Importantly, when these mutations were introduced back into wild-type Ypq1, they also block Ssh4-dependent, lysine-regulated Ypq1 degradation. As expected, coimmunoprecipitation showed that Ypq1 suppressor mutants have reduced affinity to Ssh4. Since the cytosolic loop contains a previously known Rsp5 recognition motif, they further characterized the role of Ypq1 TMDs in ligase recruitment.

Structural modeling suggests that TM5 and TM7 are juxtaposed to each other. Systematic mutagenesis targeting each residue of these two TMDs further consolidated the residues essential for Ssh4-mediated degradation. A similar mutagenesis study on Ssh4 revealed an important role for the Ssh4 TMD in Ypq1 degradation. Interestingly, for both Ssh4 and Ypq1, many identified residues are clustered on one side of the membrane helices. Arines et al. propose that Ssh4 uses its TMD to recognize TM5 and TM7 in Ypq1 based on a charge complementation experiment: a charged residue introduced into TM5 of Ypq1 abolished Ssh4-mediated degradation, but introducing an opposite charge into the TMD of Ssh4 restored lysine-regulated Ypq1 degradation.

The recognition of Ypq1 by Ssh4 appears to occur when Ypq1 adopts a specific conformation during lysine transport because charge complementarity–based degradation of Ypq1 depends on the PQ loop, which is required for lysine transport. Additionally, structural modeling of Ypq1 suggested that in the inward-open and occluded conformations, TM5 is packed against TM7, but the two TMDs become distant from each other in the outward-open conformation, which exposes residues critical for Ssh4 recognition. These findings suggest that the rapid conformational cycling during lysine transport may prevent Ssh4 recognition, but lysine depletion stalls Ypq1 in a conformation recognizable by Ssh4 (Fig. 1).

The study, together with the recent discovery of the ER membrane protein complex (EMC) in the biogenesis of multi-spanning membrane proteins at the ER, suggests a new class of chaperones that recognize specific features in TMDs. While emerging evidence suggests that the EMC recognizes exposed charged or polar residues in TMDs (10), the molecular basis of Ssh4 substrate interaction remains unclear. Like cytosolic chaperones, the EMC at the ER appears to play a dual role: while initially shielding charged/polar residues to facilitate TMD assembly, it may eventually target misassembled membrane proteins for degradation. By contrast, TMD-specific chaperones in other organelles like Ssh4 may have a more dedicated function in PQC. Clearly, more TMD-specific chaperones await to be discovered. Additionally, future studies will surely reveal not only the range of substrates and functions for each TMD-specific chaperone but also the structural basis of TMD recognition.

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