Molecular Cell, Vol. 19, 717–726, September 16, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.08.029

Previews

Yos9p: A Sweet-Toothed Bouncer of the Secretory Pathway

In this issue of *Molecular Cell*, Bhamidipati et al. (2005), Kim et al. (2005), and Szathmary et al. (2005) demonstrate that Yos9p selectively binds to aberrant glycoproteins in the endoplasmic reticulum (ER) and targets them for destruction through the ER-associated protein degradation pathway.

The degradation of nonnative glycoproteins in the secretory pathway involves the recognition of the defect in the ER lumen and the dislocation of the substrate to the cytosol where the protein is degraded by the proteasome through a process termed ER-associated protein degradation or ERAD (Ahner and Brodsky, 2004). Over the past decade, a more thorough understanding of the cytosolic ERAD events has emerged, which include extraction of the ERAD substrate by the AAA-ATPase Cdc48p, ubiquitination by E3 ligases, removal of glycans by the endoglycosidase Pngase, and the subsequent degradation by the proteasome (Figure 1). However, many questions still remain about the upstream lumenal and intermembrane ERAD processes. For example, how are terminally misfolded proteins selected for ERAD from a background of maturing and matured proteins? Recent studies from the Weissman, Ng, and Jakob groups identify Yos9p as a yeast protein that appears to act as a quality control receptor recognizing and targeting aberrant glycoproteins to the ERAD pathway, shedding much needed light on the mechanism of ERAD substrate selection (Bhamidipati et al., 2005; Kim et al., 2005; Szathmary et al., 2005).

N-linked glycans act as maturation and quality control tags in the early secretory pathway (Helenius and Aebi, 2004; Hebert et al., 2005). Their bulky and hydrophilic nature makes them highly accessible to glycosidases and transferases that remodel their structure as well as carbohydrate binding proteins that bind glycans of specific compositions and aid in the folding or sorting of glycoproteins in the ER. The generation of a degradation signal involves mannose trimming, which creates a signal that is likely decoded by a mannose-specific binding protein that sorts glycoproteins for ERAD (Cabral et al., 2001). The yeast Yos9 protein (Yos9p) possesses many of the properties expected of such a quality control receptor. Yos9p is a soluble glycoprotein, which is retained in the ER through its C-terminal HDEL sequence (Kim et al., 2005). All three studies along with a previously published paper by the Wolf group demonstrate that the deletion of *yos9* stabilizes the expression of both soluble and membrane-glycosylated ERAD substrates, whereas its absence has no effect on the stability of nonglycosylated ERAD substrates (Buschhorn et al., 2004; Bhamidipati et al., 2005; Kim et al., 2005; Szathmary et al., 2005).

Previous work has shown that there are two distinct mechanisms in the ER for monitoring and identifying ERAD substrates (Vashist and Ng, 2004). ERAD-lumenal (ERAD-L) machinery identifies cargo with defects that reside in the ER lumen. In contrast, membrane proteins with defects on cytosolic portions of an ERAD substrate are sorted through the ERAD-cytosolic (ERAD-C) mechanism. Yos9p specifically promotes the degradation of ERAD-L substrates (Bhamidipati et al., 2005; Kim et al., 2005).

Central to the function of Yos9p is its mannose 6-phosphate receptor homology (MRH) domain (Munro, 2001). MRHs are involved in glycan recognition, and five of the nine signature residues of an MRH are conserved in Yos9p. Point mutations in these conserved residues, which comprise its sugar binding pocket, disrupt the ability of Yos9p to accelerate the degradation of ERAD substrates, indicating that the lectin activity of Yos9p is essential for its role in ERAD (Bhamidipati et al., 2005; Szathmary et al., 2005).

To explore the binding specificity for Yos9p, affinity tags were added to the C terminus of Yos9p for use in pull-down assays. Here, Yos9p binding studies by both the Weissman and Jakob groups produced conflicting results. The Jakob group generated misfolded substrates containing glycans with varying compositions using yeast strains defective in glycan assembly to demonstrate that Yos9p bound only to glycoproteins possessing Man8 or Man5 glycans (Szathmary et al., 2005). This finding is in agreement with the necessity of mannose trimming for efficient degradation of glycosylated substrates. In sharp contrast, the Weissman group found that Yos9p binding was glycan independent, because (1) binding persisted after the deglycosylation of the substrate with an endoglycosidase, (2) more pronounced substrate binding was observed when the sugar binding site of Yos9p was mutated, and (3) Yos9p efficiently bound to an ERAD substrate with all of its glycosylation sites deleted (Bhamidipati et al., 2005).

Native proteins are also trimmed of mannose residues prior to their export from the ER. A degradation signal likely requires an additional component besides mannose trimming to distinguish defective proteins from native secretory cargo. Therefore, a quality control receptor may recognize both the glycan signal and the nonnative protein region directly (Figure 1). A protein recognition property could be similar to that of traditional molecular chaperones, which bind exposed hydrophobic regions on nonnative substrates. A bipartite substrate signal was also suggested from the Ng lab studies that demonstrated the specific context of the glycan was critical for Yos9p-enhanced degradation (Kim et al., 2005). They found that only a subset of the total glycans on their model ERAD substrates was able to support Yos9p-assisted ERAD (Figure 1, black glycan). A specific order of the glycan and a nonnative domain may be dictated by the organization of the Yos9p binding sites or the enzymes that encode the degradation signal.

The possible existence of two distinct binding sites on Yos9p may help to explain the discrepancy in the

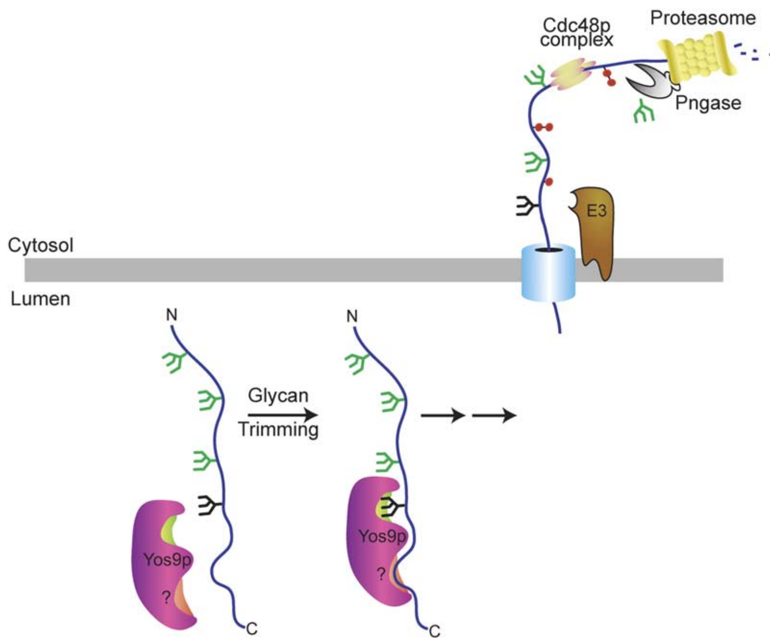

Figure 1. Yos9p Binds to ERAD Substrates and Targets them for Destruction

If a glycoprotein contains nonnative structures, its glycans are trimmed to a composition that can bind the carbohydrate binding domain of Yos9p (green binding domain). Yos9p may also possess the ability to bind to nonnative protein regions directly (orange binding site). The Yos9p bound substrate is targeted by an unknown mechanism for retrotranslocation to the cytosol where it is ubiquitinated by an E3 ligase, deglycosylated, and ultimately degraded by the proteasome.

binding studies obtained by the Weissman and Jakob groups. The Jakob group studies used a more stringent binding protocol that monitored interactions with tagged Yos9p that was expressed at physiological levels. The studies from the Weissman group relied on overexpressed protein in a strain that had deletions in downstream ERAD machinery to favor the trapping of Yos9p interactions. This less strict procedure may have al-

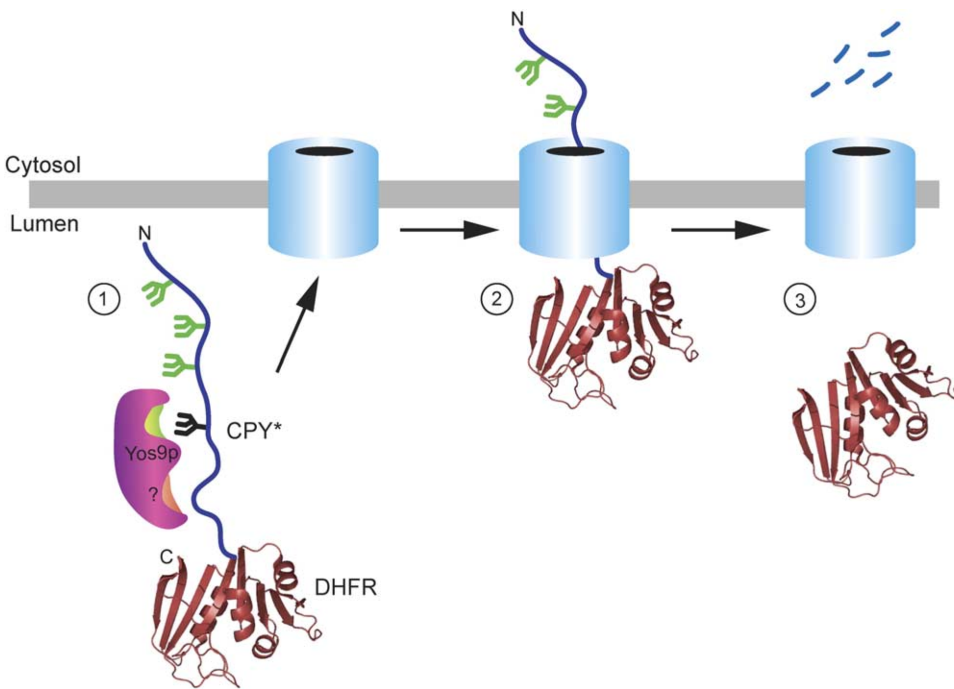

Figure 2. The Removal of Defective Glycoproteins from the ER

An ERAD substrate comprised of a stable (DHFR in red) and a misfolded protein (CPY* in blue) was employed to study the ERAD recognition and retrotranslocation processes. (1) The CPY*-DHFR chimera is bound by Yos9p prior to being targeted to a translocon (light blue) for retrotranslocation to the cytosol. (2) The stable DHFR domain traps the ERAD substrate in the translocon, slowing its subsequent degradation. (3) The trapped substrate is eventually freed by proteolysis, which releases the stable DHFR protein in the ER lumen. The site of proteolysis and the identity of the protease and the translocon are currently unknown.

lowed the visualization of weaker protein-protein interactions.

Additionally, the stable protein DHFR was attached to the termini of the ERAD substrate CPY* in an attempt to trap an ERAD substrate within the retrotranslocon (Bhamidipati et al., 2005). The degradation of CPY* was slowed when DHFR was joined to its C terminus (but not its N terminus; Figure 2). Most strikingly, the stable DHFR protein was released from the chimera in the lumen in a Yos9p-dependent manner, whereas the misfolded region became degraded. This is suggestive of a proteolytic mechanism being in place to clear retrotranslocons of ERAD proteins that possess stable modules that are difficult to unfold and translocate. This unknown protease could be cytosolic, lumenal, or localized within the membrane.

As is the case with many important findings, we are left with more questions than answers. Yos9p appears to be acting as a carbohydrate binding quality control receptor that aids in the sorting of defective proteins for ERAD. However, does Yos9p binding require a dual signal? Is binding controlled by ATP, as is the case with traditional chaperones? Once Yos9p binds a protein, how does it help in the ERAD process? Does it maintain the ERAD substrate in a translocation-competent state or does it deliver the defective protein to the retrotranslocon? Is Yos9p part of a larger functional complex? What is the nature of the ERAD retrotranslocon and the protease that clears the clogged retrotranslocons? Future studies will be needed to fully understand the role Yos9p plays in ERAD.

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