

Rot1 is degraded by ERAD: new insights into protein homeostasis of an essential gene

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Rot1, an essential type-I ER (endoplasmic reticulum) membrane protein, is implicated in diverse functions as α -1,6 glycan synthesis, actin cytoskeleton polarization dynamics, lysis of autophagic bodies and most importantly as a molecular chaperone [1-4]. For these varied roles, Rot1p adopts an unusual topology whereby the N-terminus, which gets glycosylated, faces the ER lumen and its single hydrophobic C-terminus with its essential serine-250 residue enables cell survivability [5, 6]. In addition, a comprehensive genetic-interaction analysis suggested its interaction with the proteasomal system. Whilst its diverse functions have been extensively studied; the dynamics of Rot1 protein stability in yeast cells is unknown. In this [issue](#), Juanes et.al., [doi.org/cg8p] showed that the Rot1 protein stability is mediated by the ubiquitin proteasome system (UPS), which is dependent on the main degradation pathway located at the ER, the ER-associated degradation system (ERAD). Here the authors employed the GAL4 shut-off assay to ascertain the role of the ERAD system, Ubc6, Ubc7 and Hrd1, along with Pre1 and Pre2 of the UPS on Rot1 protein stability.

Typically, ERAD system targets misfolded proteins within the ER for ubiquitination to be degraded by the proteasome complex, but this system has now been recognized to degrade proteins to maintain their optimal levels in cells. By employing its three conserved processes—E1, E2 and E3—target proteins are ubiquitylated and targeted for degradation. In the budding yeast, Uba1 (the E1 enzyme); Ubc1, Ubc6 and Ubc7 (of the E2 enzymes) and, Hrd1/Der3 and Doa10 (of the E3 enzyme

processes) recognize degron sequences of its different substrate proteins [7-10]. Based on the specific substrate domain that is assessed, the ERAD system is categorized as the ERAD-C (cytoplasmic domain), the ERAD-L (luminal domain) and the ERAD-M (membrane domain), all of which allows easy identification of proteins with misfolded proteins [11]. For successful transport of the ubiquitinated protein to the cytoplasm for degradation by the UPS, the ubiquitin binding factor, Cdc48p-Npl4p-Ufd1p complex plays a critical role [10]. The final step of degradation of the ubiquitinated proteins takes place in the 26S proteasome, composed of the 19S and 20S particles along with the chymotryptic factors Pre1, Pre2 and Pre4, which together are essential for the degradation of the ubiquitinated protein [12].

Juanes et.al., conducted systematic experiments that measures protein levels of Rot1 in a 60 minute interval in different mutant backgrounds, and concluded that the ERAD system proteins—Ubc6, Ubc7 and Hrd1—and the UPS proteins—Pre1 and Pre2—are all required for maintaining accurate levels of this essential gene. This study confirms the genetic analysis results that the authors had previously published. It would be interesting to see if other proteins in the ERAD and UPS systems would also effect Rot1 levels, and likewise if human Rot1 levels were similarly altered in the studied mutant backgrounds.

1. Pasikowska M, Palamarczyk G, Lehle L. The essential endoplasmic reticulum chaperone Rot1 is required for protein N- and O-glycosylation in yeast. *Glycobiology*. 2012;22(7):939-47. doi:

- 10.1093/glycob/cws068. PubMed PMID: 22492205.
2. Takeuchi M, Kimata Y, Kohno K. *Saccharomyces cerevisiae* Rot1 is an essential molecular chaperone in the endoplasmic reticulum. *Mol Biol Cell*. 2008;19(8):3514-25. doi: 10.1091/mbc.E07-12-1289. PubMed PMID: 18508919; PubMed Central PMCID: PMC2488298.
 3. Machi K, Azuma M, Igarashi K, Matsumoto T, Fukuda H, Kondo A, et al. Rot1p of *Saccharomyces cerevisiae* is a putative membrane protein required for normal levels of the cell wall 1,6-beta-glucan. *Microbiology*. 2004;150(Pt 10):3163-73. doi: 10.1099/mic.0.27292-0. PubMed PMID: 15470097.
 4. Juanes MA, Queralt E, Bano MC, Igual JC. Rot1 plays an antagonistic role to Clb2 in actin cytoskeleton dynamics throughout the cell cycle. *J Cell Sci*. 2007;120(Pt 14):2390-401. doi: 10.1242/jcs.002758. PubMed PMID: 17606994.
 5. Juanes MA, Carlos Igual J, Carmen Bano M. Membrane topology and post-translational modification of the *Saccharomyces cerevisiae* essential protein Rot1. *Yeast*. 2008;25(2):93-106. doi: 10.1002/yea.1549. PubMed PMID: 17914748.
 6. Juanes MA, Martinez-Garay CA, Igual JC, Bano MC. Targeting and membrane insertion into the endoplasmic reticulum membrane of *Saccharomyces cerevisiae* essential protein Rot1. *FEMS Yeast Res*. 2010;10(6):639-47. doi: 10.1111/j.1567-1364.2010.00653.x. PubMed PMID: 20608986.
 7. McGrath JP, Jentsch S, Varshavsky A. UBA 1: an essential yeast gene encoding ubiquitin-activating enzyme. *EMBO J*. 1991;10(1):227-36. PubMed PMID: 1989885; PubMed Central PMCID: PMC452634.
 8. Lemus L, Goder V. Regulation of Endoplasmic Reticulum-Associated Protein Degradation (ERAD) by Ubiquitin. *Cells*. 2014;3(3):824-47. doi: 10.3390/cells3030824. PubMed PMID: 25100021; PubMed Central PMCID: PMC4197631.
 9. Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nat Cell Biol*. 2001;3(1):24-9. doi: 10.1038/35050524. PubMed PMID: 11146622.
 10. Thibault G, Ng DT. The endoplasmic reticulum-associated degradation pathways of budding yeast. *Cold Spring Harb Perspect Biol*. 2012;4(12). doi: 10.1101/cshperspect.a013193. PubMed PMID: 23209158; PubMed Central PMCID: PMC3504435.
 11. Kawaguchi S, Ng DT. SnapShot: ER-associated protein degradation pathways. *Cell*. 2007;129(6):1230. doi: 10.1016/j.cell.2007.06.005. PubMed PMID: 17574032.
 12. Seufert W, Jentsch S. In vivo function of the proteasome in the ubiquitin pathway. *EMBO J*. 1992;11(8):3077-80. PubMed PMID: 1322295; PubMed Central PMCID: PMC4556791.