Sterol homeostasis requires regulated degradation of squalene monooxygenase by the ubiquitin ligase Doa10/Teb4

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Figure 1. Erg1 is a substrate of the Doa10 complex. (A) Schematic representation of the mevalonate pathway and its different end products. The steps catalyzed by HMG-CoA reductase and the squalene monooxygenase Erg1 are indicated. Adapted from Goldstein and Brown (1990). (B) Erg1 abundance in the indicated mutants relative to wt cells, as detected by mass spectrometry upon SILAC labeling. All strains used are lysine auxotrophs and were grown in the presence of either heavy L-lysine (wt cells) or light L-lysine (deletion mutants). Note that high steady state levels of Erg1 result in a low heavy/light ratio. (C) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt cells or in cells with the indicated deletions. Whole-cell extracts were analyzed by SDS–PAGE and western blotting. Erg1 was detected with α-Erg1 antibody. Phosphoglycerate kinase (Pgk1) was used as loading control and detected with α-Pgk1 antibodies. A representative gel of three independent experiments is shown. (D) The degradation of endogenous Erg1 was analyzed as in (C) in wt cells or the temperature sensitive cdc48-3 and npl4-1 cells either at the permissive temperature of 25°C or after a 2 hr shift to 37°C, the restrictive temperature.

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Figure 1—figure supplement 1. Abundance of Erg1 but not of other components of the Erg pathway is altered in Doa10 complex mutants. (A) Abundance of the indicated Erg pathway components in endoplasmic reticulum-associated protein degradation (ERAD) mutants relative to wt cells, as detected by mass spectrometry upon SILAC labeling. All strains used are lysine auxotrophs and were grown in the presence of either heavy L-lysine (wt cells) or light L-lysine (deletion mutants). Only proteins in which more than one peptide was detected by mass spectrometry were included. Note that high steady state levels of a protein result in a low heavy/light ratio. (B) The degradation of Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt cells or in cells with the indicated deletions. Expression of Erg1 was driven from the strong constitutive promoter glyceraldehyde-3-phosphate dehydrogenase (GAPDH) replacing the endogenous ERG1 promoter. Whole-cell extracts were analyzed by SDS–PAGE and western blotting. Erg1 was detected with α-Erg1 antibody. Phosphoglycerate kinase (Pgk1) was used as loading control and detected with α-Pgk1 antibodies. A representative gel of three independent experiments is shown.
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Figure 2. Doa10-dependent degradation of Erg1 depends on a single lysine residue. (A) Expression of ERG1 or ERG1-derivatives with the indicated lysine mutations from a plasmid rescues the growth of yeast cells upon repression of the endogenous ERG1. A yeast strain expressing endogenous ERG1 from the regulated GAL1 promoter was transformed with plasmids encoding different lysine mutants or a control empty vector. The growth of serial dilutions of cells was tested under conditions of induced (galactose-containing media) or repressed (glucose-containing media) endogenous ERG1. (B) The degradation of Erg1 or the indicated Erg1 lysine mutant expressed from the endogenous ERG1 promoter was followed after inhibition of protein synthesis by cycloheximide in wt or doa10Δ cells. Samples were analyzed as in Figure 1C. (C) Quantitation of two independent experiments performed as described in (B).

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Figure 2—figure supplement 1. Doa10-dependent degradation of Erg1 depends on the lysine residue at position 311. (A) The degradation of Erg1 or the indicated Erg1 lysine mutants expressed from the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter was followed after inhibition of protein synthesis by cycloheximide. Samples were analyzed as in Figure 1C.
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Figure 3. Flux through the sterol pathway regulates Erg1 degradation. (A) Schematic representation of the ergosterol biosynthetic pathway highlighting the enzymatic steps affected by the small inhibitor zaragozic acid, Ro48-807, and fluconazole. The step catalyzed by the squalene monooxygenase Erg1 is also indicated. (B) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or wt cells treated for 2 hr with 10 µg/ml zaragozic acid (ZA). Samples were analyzed as described in Figure 1C. (C) Quantitation of three independent experiments as described in (B) in cells expressing the Doa10 substrate Vma12-Ndc10_{902-956}-HA, a model misfolded protein. Vma12-Ndc10_{902-956}-HA was detected with anti-HA antibodies. (D) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or wt cells treated for 2 hr with 40 µg/ml Ro48-807 (Ro48). Samples were analyzed as described in Figure 1C. (E) Quantitation of three independent experiments as described in (D) in cells expressing the Doa10 substrate Vma12-Ndc10_{902-956}-HA, a model misfolded protein. Vma12-Ndc10_{902-956}-HA was detected with anti-HA antibodies. (F) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt and doa10Δ cells. Cells were incubated for 1 hr with DMSO or with 10 µg/ml fluconazole (Fluco). Samples were analyzed as Figure 3. Continued on next page
described in Figure 1C. (G) Quantitation of three independent experiments as described in (F) in cells expressing the Doa10 substrate Pca1\textsubscript{1–392}-DHFR-HA, a model misfolded protein. Pca1\textsubscript{1–392}-DHFR-HA was detected with anti-HA antibodies. (H) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt cells or in cells with the indicated mutations. Samples were analyzed as described in Figure 1C. DOI: 10.7554/eLife.00953.007

Figure 3—figure supplement 1. Sterol depletion affects Doa10-dependent degradation of Erg1 but not of other Doa10 substrates. (A) The degradation of the Doa10 substrates Vma12-Ndc10\textsubscript{902–956}-HA and Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or in wt cells treated for 2 hr with 10 µg/ml zaragozic acid (ZA). Samples were analyzed as described in Figure 1C. Vma12-Ndc10\textsubscript{902–956}-HA was detected with anti-HA antibodies. (B) The degradation of the Doa10 substrates Pca1\textsubscript{1–392}-DHFR-HA and Erg1 was followed as in (A). (C) Quantitation of three independent experiments as described in (A) in cells expressing the Doa10 substrate Pca1\textsubscript{1–392}-DHFR-HA. (D) The degradation of the Doa10 substrates Vma12-Ndc10\textsubscript{902–956}-HA and Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or in wt cells treated for 2 hr with 40 µg/ml Ro48-807 (Ro48). Samples were analyzed as described in Figure 1C. Vma12-Ndc10\textsubscript{902–956}-HA was detected with anti-HA antibodies. (E) The degradation of the Doa10 substrates Pca1\textsubscript{1–392}-DHFR-HA and Erg1 was followed as in (D). (F) Quantitation of three independent experiments as described in (D) in cells expressing the Doa10 substrate Pca1\textsubscript{1–392}-DHFR-HA. (G) The degradation of the Doa10 substrates Vma12-Ndc10\textsubscript{902–956}-HA and Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or in cells treated for 1 hr with 10 µg/ml fluconazole (Fluco). Samples were analyzed as described in Figure 1C. (H) The degradation of the Doa10 substrates Vma12-Ndc10\textsubscript{902–956}-HA and Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt control cells (DMSO) or in cells treated for 1 hr with 10 µg/ml fluconazole (Fluco). Samples were analyzed as described in Figure 1C. (I) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in wt cells or in cells with the indicated mutations. Samples were analyzed as described in Figure 1C. DOI: 10.7554/eLife.00953.008
Figure 4. Doa10-dependent degradation of Erg1 affects sterol homeostasis and, together with sterol esterification, is essential to prevent buildup of sterol intermediates. (A) Relative amounts of ergosterol, lanosterol, and ergostadienol in cells with the indicated genotype. Cells were grown in synthetic complete (SC) media until early stationary phase, and lipids were extracted and analyzed by shotgun lipidomics. (B) Analysis of sterol esters in \( \text{wt} \) and \( \text{doa10}\Delta \) cells by mass spectrometry. Cells were grown in SC media until early stationary phase, and lipids were extracted and analyzed by shotgun lipidomics. (C) Serial dilutions of cells with the indicated genotype were spotted on YPD or YPD + 0.3% of benzyl alcohol (BA) and incubated for 2 (25°C), 4 (YPD + 0.3% BA) or 5 days (14°C). (D) Serial Figure 4. Continued on next page
dilutions of cells with the indicated genotype were spotted on YPD and incubated for 2 (25°C) or 5 days (14°C). (E) The degradation of endogenous Erg1 was followed after inhibition of protein synthesis by cycloheximide in cells with the indicated genotype. Samples were analyzed as described in Figure 1C. (F) The degradation of Erg1 or the indicated Erg1 lysine mutants expressed from the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter was followed after inhibition of protein synthesis by cycloheximide in wt or are1Δ are2Δ cells. Samples were analyzed as described in Figure 1C.

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Figure 4. Continued

Figure 5. Doa10 homologue Teb4 promotes degradation of human squalene monooxygenase. (A) The degradation of endogenous squalene monooxygenase (SM) was followed after inhibition of protein synthesis by cycloheximide in sterol-deprived HEK293 cells treated with control siRNA or siRNA targeting the ubiquitin ligase Teb4. The degradation of SM was monitored under basal conditions (methyl-β-cyclodextrin vehicle, +CD) and upon addition of cholesterol (+CD/Ch). Where indicated DMSO or the proteasome inhibitor MG132 (10 µM) was included. Samples were analyzed by SDS–PAGE and immunoblotting. SM was detected with rabbit polyclonal anti-SM antibodies and α-tubulin with mouse monoclonal anti-α-tubulin antibodies. The arrow indicates the band corresponding to SM. (B) Quantitation of three independent experiments performed as described in (A).

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**Figure 5—figure supplement 1.** Overexpression of dominant negative Teb4(C9A), but not of wt Teb4, strongly delays degradation of human squalene monoxygenase. The degradation of endogenous squalene monoxygenase (SM) was followed after inhibition of protein synthesis by cycloheximide in sterol-deprived HEK293 cells transfected with Teb4-myc or Teb4(C9A)-myc. The degradation of SM was monitored under basal conditions (methyl-β-cyclodextrin vehicle, +CD) and upon addition of cholesterol (+CD/Ch). Where indicated, DMSO or the proteasome inhibitor MG132 (10 µM) was included. Samples were analyzed by SDS–PAGE and immunoblotting. SM was detected with rabbit polyclonal anti-SM antibodies and α-tubulin with mouse monoclonal anti-α-tubulin antibodies. The arrow indicates the band corresponding to SM.
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Figure 6. A central role of endoplasmic reticulum-associated protein degradation in sterol homeostasis. (A) Schematic representation of the feedback inhibition systems required for sterol homeostasis in yeast (left) and mammals (right) previously characterized (dotted lines) and described here (solid lines). Endoplasmic reticulum-associated protein degradation (ERAD) ubiquitin ligases are in bold and the enzymes targeted by ERAD-regulated degradation are enclosed in gray boxes. DOI: 10.7554/eLife.00953.012