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N-terminal HEAT repeats of *C. albicans* Tor1 are required for specific stress responses and for down-modulating TORC1 signaling activity

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Abstract

Persistence and growth of *C. albicans* as a commensal or pathogen necessitates resistance to nutritional, oxidative, heat and other stressors, imposed by the host immune system or the competing bacterial microbiome. The Target of Rapamycin (TOR) signaling pathway controls eukaryotic cell growth and proliferation. We hence predicted *C. albicans* TOR signaling to play a central role in responding to these stressors. Tor1 kinase has several large protein-protein interaction domains, among which the largest proportion consists of Huntingtin, Elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and TOR1 (HEAT) repeats. Which regions of Tor1 respond to specific stressors has not been defined. We constructed strains in which the only *TOR1* allele, controlled by repressible *tetO*, encodes either a full-length Tor1 (*TOR1-full*) or a truncated Tor1 (*TOR1-trunc*) that lacks 381 N-terminal amino acid residues comprising ~10 HEAT repeats. Compared to controls, growth of cells expressing *TOR1-trunc* was strongly defective during oxidative-, cell wall- or heat stress, but not during plasma membrane stress. TOR Complex 1 (TORC1) activity was abnormally elevated in cells expressing *TOR1-trunc* during stress responses, indicating N-terminal HEAT repeats of Ca.Tor1 are required for down-regulation of TORC1 activity and for resistance to specific stressors.

Introduction

The Target of Rapamycin (TOR) signaling pathway controls growth and proliferation in all eukaryotes by integrating nutritional information, as well as participating in stress and survival responses [1]. Models of the budding yeast Tor1 kinase show that its 20 N-terminal HEAT repeats are exposed at the surface of TORC1 and accessible to interaction with regulatory molecules [2].

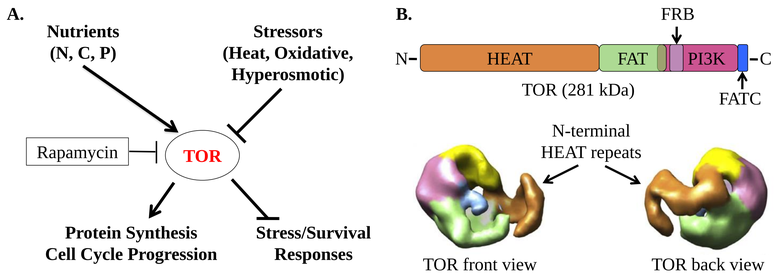


Fig. 1. Target of Rapamyin (TOR) Signaling pathways and TOR structure
A. Tor kinase is the central node of the TOR singling pathway. It up-regulates anabolic processes (cell growth and proliferation) and down-regulates catabolic processes (autophagy, proteolysis), and is inhibited by the anti-fungal and immunosuppressive agent rapamycin. B. Schematic representation and 3D EM map (25Å) of yeast TOR1, N-terminal HEAT repeats are denoted by orange colour [2].

Summary

- Ca.Tor1 N-terminal HEAT repeats are required for down-regulation of TORC1 activity.
- Improper TORC1 activation is associated with a growth defect under specific stress conditions, possibly due to the inability to activate the HOG stress-response signaling pathway.
- Resistance to certain stressors (eg. plasma membrane stress) is independent of TORC1 down-modulation.
- Numerous components of TORC1 anabolic signaling activity contribute to specific types of stress responses, highlighting the importance of TORC1 in responding not only to nutritional stress (starvation) but also to other conditions that threaten cellular homoeostasis.

Reference

1. Loewith R, Hall MN. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* (2011) 189:1177-1201.
2. Adami A, *et al.* Structure of TOR and its complex with KOG1. *Mol Cell* (2007) 27:509-516.

Results

1. HEAT repeats are required to down-regulate TORC1 activity during N starvation.

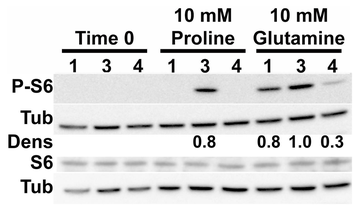


Fig. 2. Western blotting assay of Rps6 phosphorylation (P-S6) in *tetO-TOR1* strains grown in non-preferred (proline) and preferred (glutamine) nitrogen sources.
Cells of indicated genotypes were grown in *tetO*-suppressing medium before releasing into permissive medium with poor or preferred nitrogen sources and grown for 45 minutes.
Dens: densitometry of P-S6 vs. Tubulin (Tub). S6: total Rps6.

2. Loss of HEAT repeats leads to hypersensitivity to Oxidative Stress (OS).

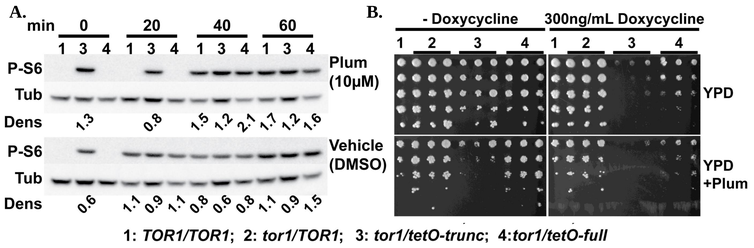


Fig. 3 TORC1 down-regulation is required in response to oxidative stress.
A. Western blots monitoring P-S6 levels. Cells of indicated genotypes were grown in *tetO* promoter non-suppressing medium before exposure to vehicle (DMSO) or plumbagin. B. Fivefold cell dilutions were spotted onto YPD media without and with the superoxide generator plumbagin (20 μM) and incubated for 2 days at 30ºC.

Decreased intracellular ROS of cells lacking Tor1 N-terminal HEAT repeats corresponds to decreased O_2 consumption and decreased HOG pathway activation.

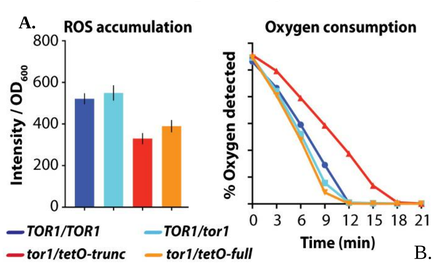
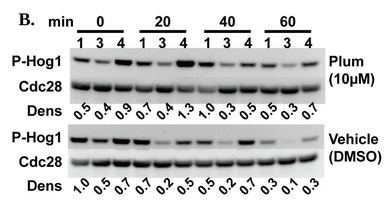


Fig. 4. *tor1/tetO-trunc* showed lower ROS accumulation, O_2 consumption and Hog1 activation.
A. Intracellular ROS (H2DCFDA detected) and O_2 consumption of *tetO-TOR1* strains. Cells of indicated genotypes were grown with 5 ng/mL doxycycline.



B. Western blots monitoring phosphorylated Hog1 (P-Hog1) levels. Cells were grown with or without 10 μM Plumbagin. Dens: densitometry of P-HOG1 vs. Cdc28.

Low baseline HOG pathway activation, possibly attributable to decreased O_2 consumption of HEAT-repeat truncated cells, may contribute to their defective oxidative stress management.

3. Loss of HEAT repeats leads to hypersensitivity to cell wall and heat stresses

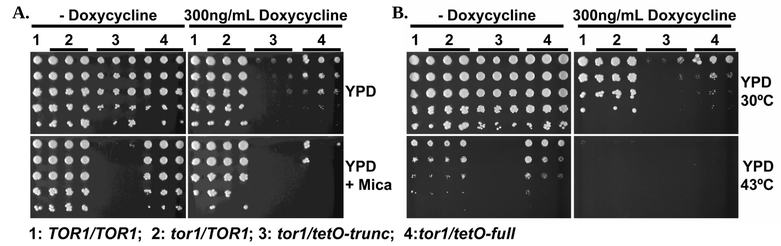


Fig. 5. Growth assay of *tetO-TOR1* strains exposed to cell wall and heat stresses.
A. Fivefold cell dilutions were spotted onto YPD media containing a cell wall stressor (10 ng/mL micafungin) and incubated for 2 days at 30ºC; B. Fivefold cell dilutions were spotted onto YPD media and incubated for 2 days at 30ºC and 43ºC, respectively.

4. Loss of HEAT repeats does not cause hypersensitivity to plasma membrane stress

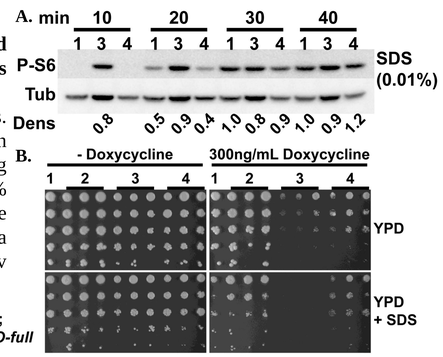


Fig. 6 P-S6 Western blotting and growth assay of *tetO-TOR1* strains exposed to plasma membrane stress.
A. Western blots monitoring P-S6 levels. Cells of indicated genotypes were grown in *tetO* promoter non-suppressing medium before exposure to SDS (0.01% w/v). B. Fivefold cell dilutions were spotted onto YPD media containing a plasma membrane stressor (0.005% w/v SDS) and incubated for 2 days at 30ºC.

5. Other TORC1 signaling pathway components participate in specific types of stress response.

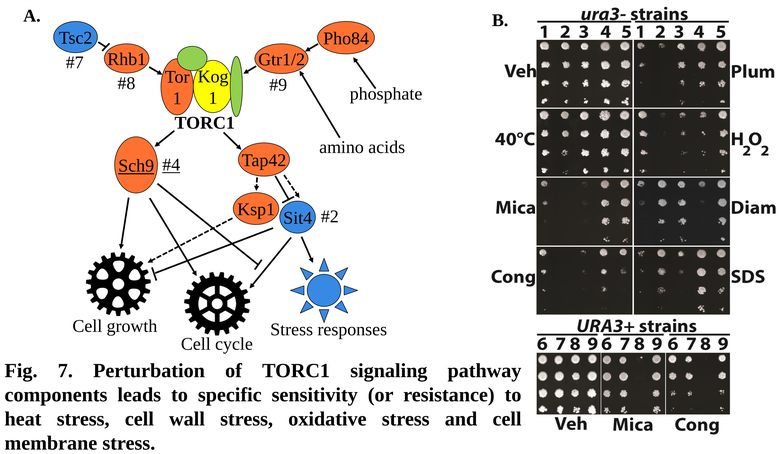


Fig. 7. Perturbation of TORC1 signaling pathway components leads to specific sensitivity (or resistance) to heat stress, cell wall stress, oxidative stress and cell membrane stress.
A. Schematic diagram of key components involved in TORC1 signaling pathway. #2, 4, 7, 8, 9 correspond to strain numbers in panel B, respectively; B. Fivefold cell dilutions were spotted onto YPD+uridine media containing different stressors and incubated for 2 days at 30°C unless specified otherwise. *Ura3-* strains: CAI4 (WT, 1), *sit4*$^{-/-}$ (2), *mds3*$^{-/-}$ (3), *sch9*$^{-/-}$ (4), *rim15*$^{-/-}$ (5), *URA3+* strains: SC5314 (WT, 6), *tsc2*$^{-/-}$ (7), *rhb1*$^{-/-}$ (8), *gtr1*$^{-/-}$ (9). Stressors: Micafungin (Mica, 20 ng/ml); Congo Red (Cong, 25 μg/ml); Plumbagin (Plum, 10 μM); Hydrogen peroxide (H_2O_2, 5 mM); Diamide (Diam, 0.75 mM); Sodium dodecyl sulfate (SDS, 0.01%); Vehicle (Veh, H_2O).