

The short-term response of yeast to potassium starvation

Barreto, L., Canadell, D., Valverde-Saubí, D., Casamayor, A., & Ariño, J. (2012). The short-term response of yeast to potassium starvation. *Environmental microbiology*, 14(11), 3026-3042.

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Transcriptomic Genome Analysis on
Potassium-Deprived Yeast Has Not Been Studied

Yeast Cells Cultured Potassium-Free YNB Based
Growth Medium

Yeast Cells Modified Gene Expression in Response to
Potassium Deprivation

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*There were no previous studies that directly correlate to this work. However, there are multiple studies that are able to supplement the work.

- Connection between potassium and ammonium when analyzing potassium
- Potassium limits growth when ammonium is the nitrogen source
- Ammonium appears to be toxic for *Saccharomyces cerevisiae*
- Ammonium toxicity is increased under high-potassium conditions
 - Caused by over-expression of ammonium transporters
- Ammonium toxicity is well-established in metazoans, but not in yeast

Potassium is the most abundant cation in the cytosol and extremely important to normal cell function

- Maintenance of normal cells functions
- Potassium homeostasis is needed across the cell membrane
- Oxidative stress and potassium imbalance can cause life threatening conditions

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Yeast cells cultured on Translucent K+ free media

- E. Coli cells cultured on LB broth
 - Contained plasmid pTRK24 (expressing TRK1 gene) or plasmids pTrk1K1147N and pTrk1M1153R (expressing mutant Trk1 gene)

Table 2. Strains used in this work.

Strain	Genotype	Source/reference
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf
BYT1	BY4741 <i>trk1Δ::loxP</i>	Navarrete <i>et al.</i> (2010)
BYT2	BY4741 <i>trk2Δ::loxP</i>	Navarrete <i>et al.</i> (2010)
BYT12	BY4741 <i>trk1Δ::loxP trk2Δ::loxP</i>	Navarrete <i>et al.</i> (2010)
BY4741 <i>rtg2</i>	BY4741 <i>rtg2::kanMX4</i>	Euroscarf
BY4741 <i>rtg3</i>	BY4741 <i>rtg3::kanMX4</i>	Euroscarf
BY4741 <i>fzo1</i>	BY4741 <i>fzo1::kanMX4</i>	Euroscarf
YNR055.1	BY4741 <i>CLN3-13MYC::KanMX4</i>	J. Clotet
YPC722 (5)	BY4741 <i>CLN1-TAP::HIS3</i>	Ghaemmaghami <i>et al.</i> (2003)
YPC723	BY4741 <i>CLN2-TAP::HIS3</i>	Ghaemmaghami <i>et al.</i> (2003)
YPC724	BY4741 <i>CLB2-TAP::HIS3</i>	Ghaemmaghami <i>et al.</i> (2003)
W303-1A	<i>MATa ade2 can1 his3 leu2 trp1 ura3</i>	R. Rothstein
DBY746	<i>MATα his3-1 leu2-3,112 ura3-52 trp1-289</i>	D. Botstein

RNA was Extracted to Perform a Microarray and RT-PCR

- Yeast cells suspended in Translucent media either with or without 50mL KCL
 - Timepoints: 10, 20, 40, 60 and 120 min
 - 4 replicates
 - 20 mL of culture at each timepoint for microarray analysis
- RNA extraction via Ribo Pure™-Yeast kit (Ambion)
 - Induction: signal ratio ≥ 2.0 -fold
 - Repression: signal ≤ 0.50 -fold.
 - RT-PCR with CIT2, DLD3, CLN2, CLN3, CLB1, CLB2, CLB5, CLB6 and ACT1 primers
 - 27-29 cycles

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Yeast Cells Modified Gene Expression in Response to Potassium Deprivation

- Short-term potassium deprivation...
 1. changes sulfur metabolism
 2. induces oxidative stress response
 3. activates the retrograde pathway.

- Associated with ammonium accumulation through the Trk1 potassium transporter.

Figure 1. The majority of the genes were induced and repressed 60-120 min after the cells were transferred to potassium free media.

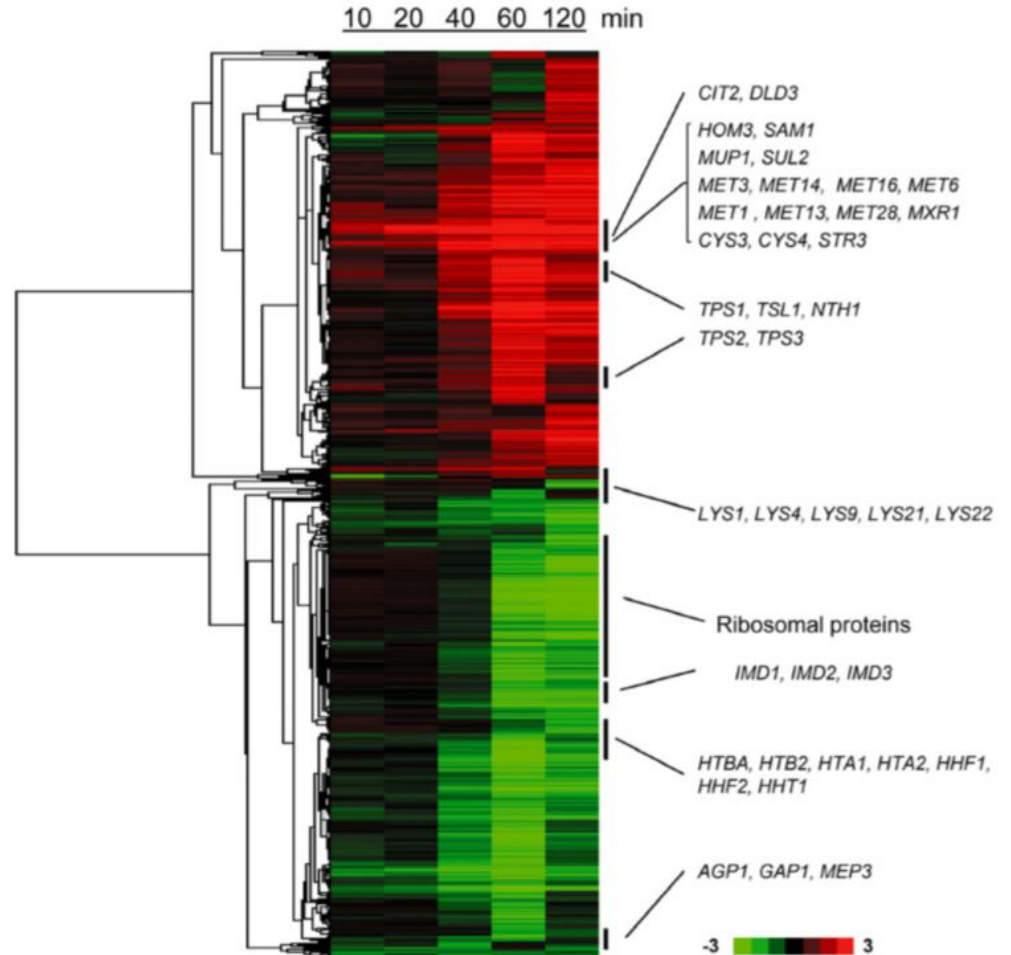


Fig. 1: Barretto et al. (2012) *Environmental microbiology*. 14:11

Table 1. The majority of the genes were induced and repressed 60-120 min after the cells were transferred to potassium free media.

Table 1. Short-term transcriptional changes associated to potassium starvation.

Genes	Minutes after transfer to K ⁺ -free medium				
	10	20	40	60	120
Induced	53	58	255	568	619
Repressed	45	33	235	733	560

Figure 2A. Potassium starvation decreases sulfuric amino acid metabolism.

- Model demonstrates the sulfur uptake and how Met/Cys are metabolized.

A)

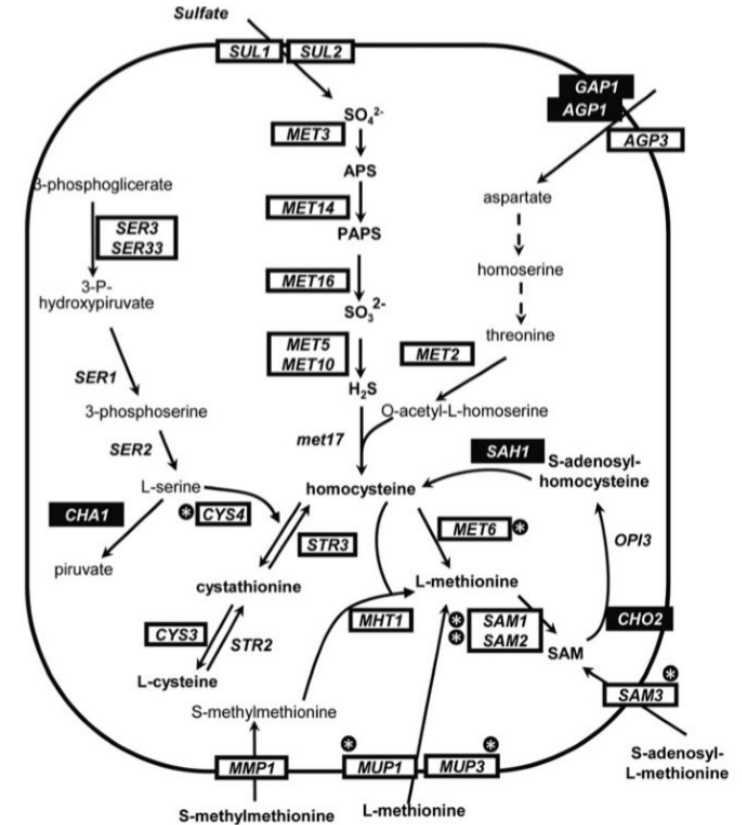


Fig. 2A: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 2B. Potassium starvation decreases sulfuric amino acid metabolism.

- Graph depicting relative concentrations of mRNA for genes associated with Met/Cys metabolism
- Increase in expression of genes → depletion of Met/Cys in cells

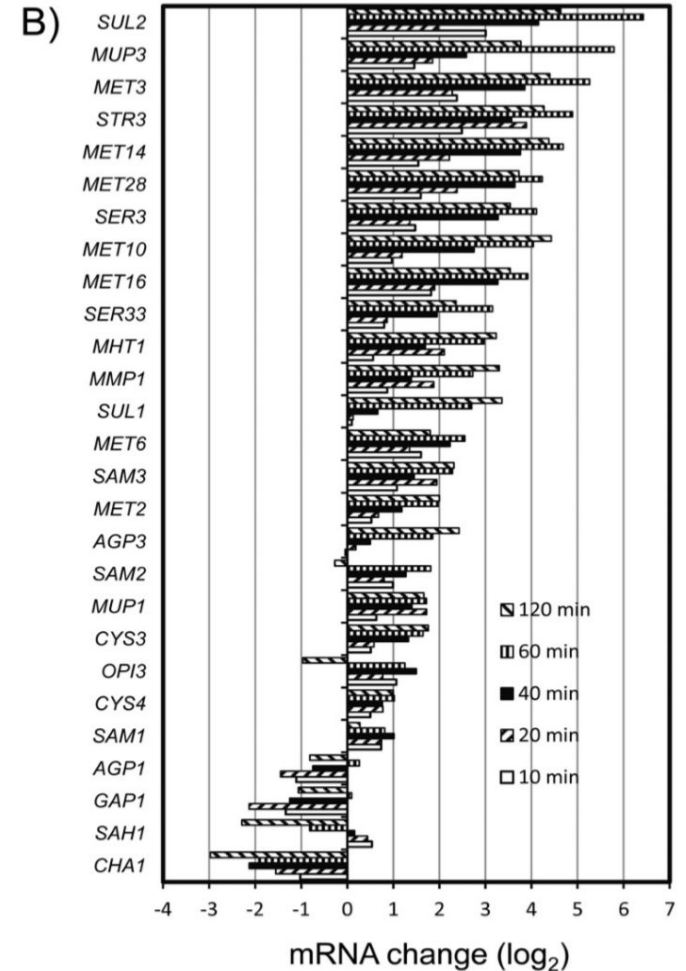


Fig. 2B: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 2C. Potassium starvation decreases sulfuric amino acid metabolism.

- Relative concentration of Met/Cys after treatment
- The concentration of Met/Cys amino acids decreases with potassium treatment

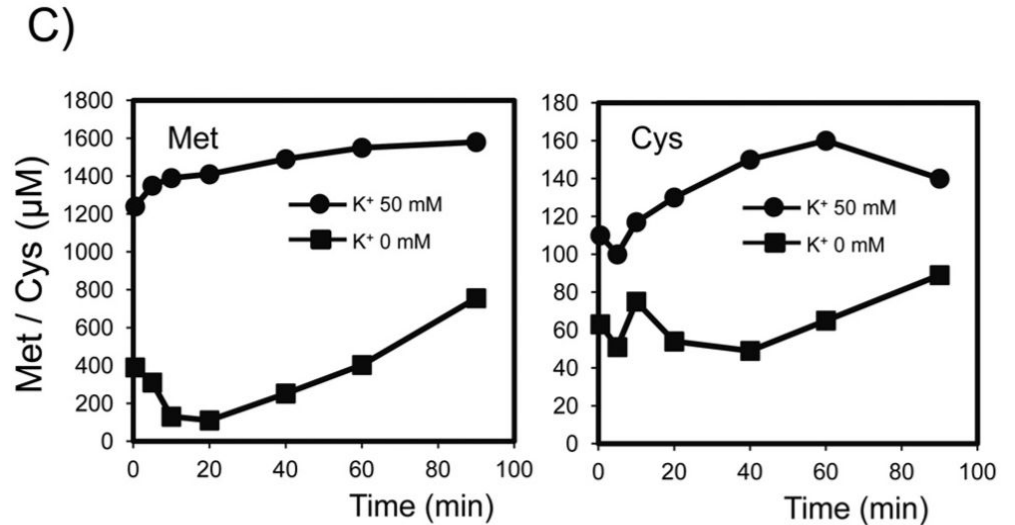


Figure 3A: Oxidative Stress Response Genes Are Induced by Potassium Deprivation

- After potassium starvation
- Measured through microarray experiments

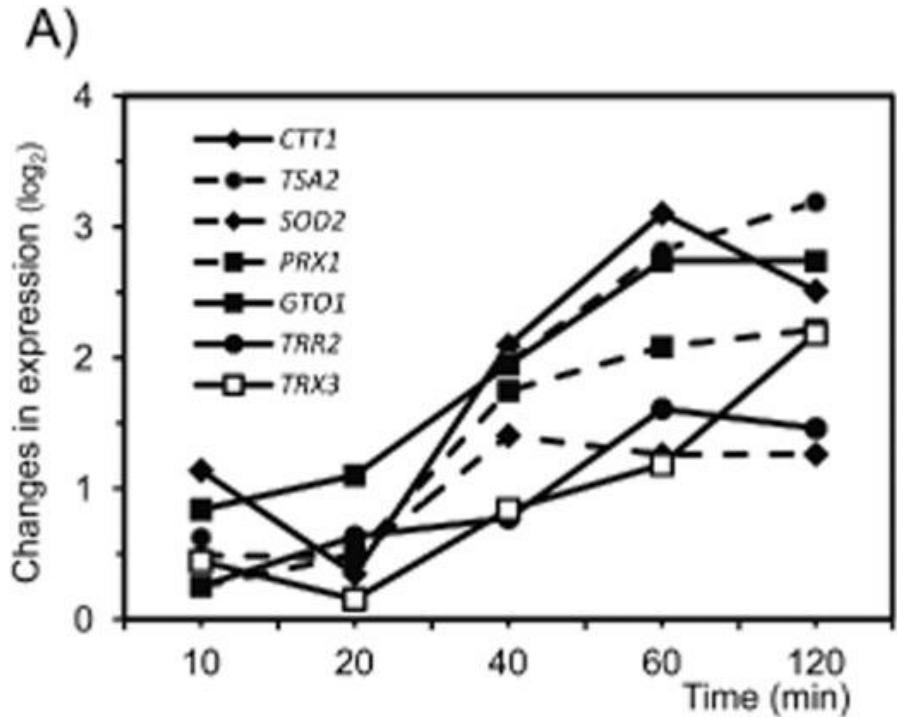


Figure 3B: Oxidative Stress Response Genes Are Induced by Potassium Deprivation

- Oxidation of Dye
- Fluorescent indicates oxidation
- ROS formation causes oxidation when switch to K^+ -free medium

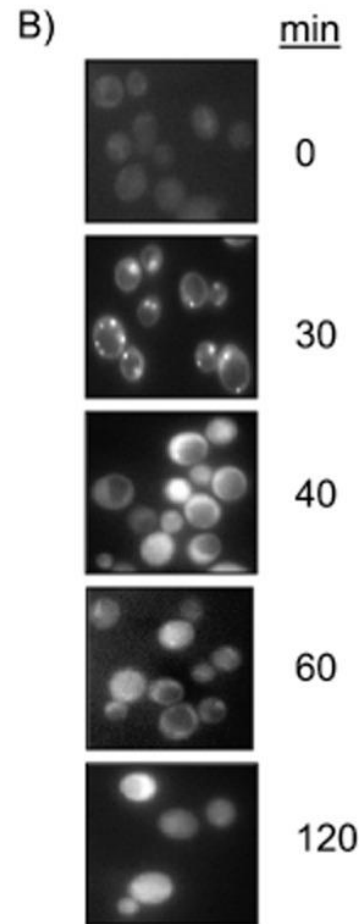


Figure 3C: Oxidative Stress Response Genes Are Induced by Potassium Deprivation

- Glutathione Levels
- Glutathione important to redox balance
- Reduced form (square) - GSH
Oxidized form (circle) - GSSG
- Potassium starvation- decline in GSH and increase in GSSG
- Overall decrease in ratio

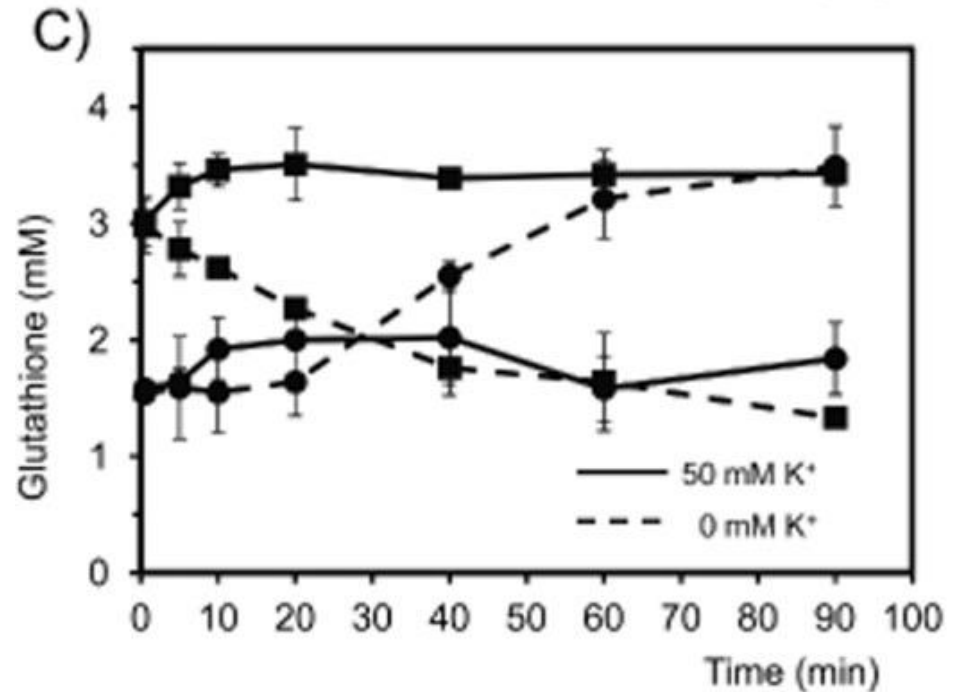


Figure 4A: Methylglyoxal Increases with Trehalose Metabolism

- Mechanism of MG
- Methylglyoxal (MG) byproduct of glycolysis
- MG detoxification
- MG initiation of trehalose metabolism

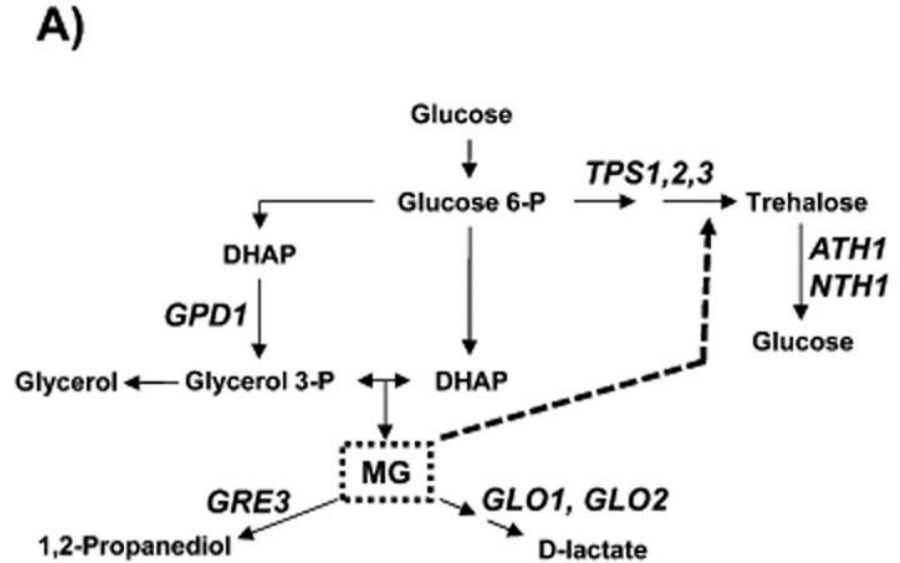


Figure 4B: Methylglyoxal Increases with Trehalose Metabolism

- Genes in trehalose synthesis
- Genes involved in trehalose synthesis show an increase in expression
- Increase in methylglyoxal shown by increased expression of GLO1 and GLO2

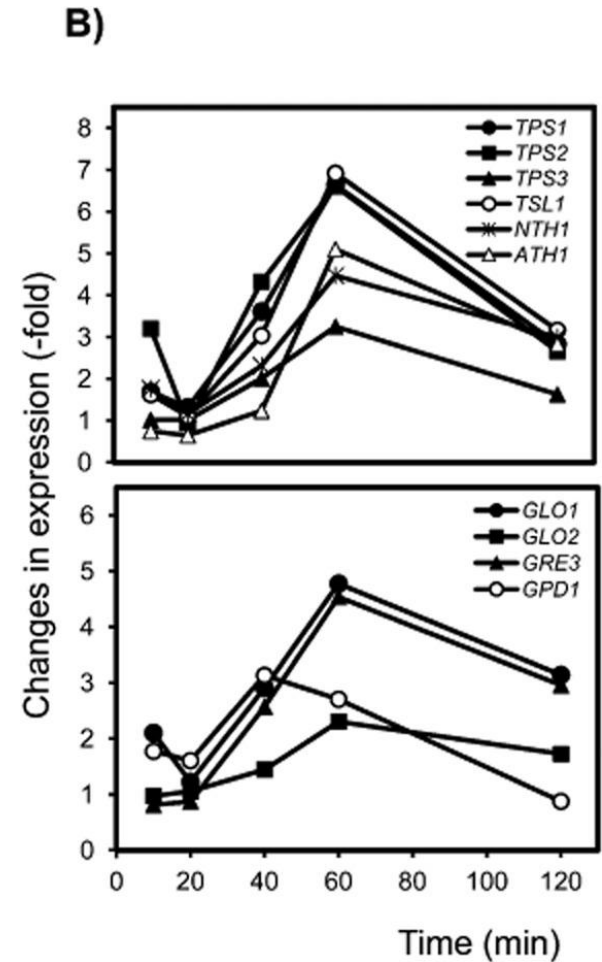


Fig. 4B: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 4C: Methylglyoxal Increases with Trehalose Metabolism

- Resuspended without potassium (----) or with 50 mM potassium (-)
- Genes in MG detoxification enhanced without potassium
- Genes linked to trehalose metabolism genes

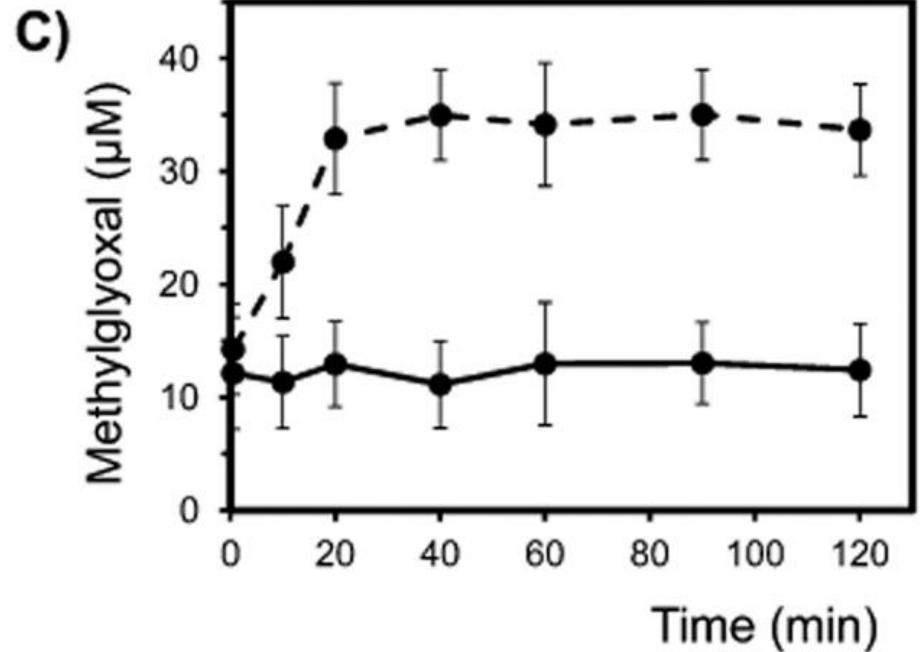


Figure 5A. Potassium starvation leads to a strong increase in expression of CIT2 & DLD3 genes central to the retrograde response

- The retrograde response involves the transmission of information from mitochondria (in eukaryotes other than plants) to the nucleus
- Deletion of RTG3 results in blockage of the increased expression of CIT2 and DLD3 in response to potassium starvation

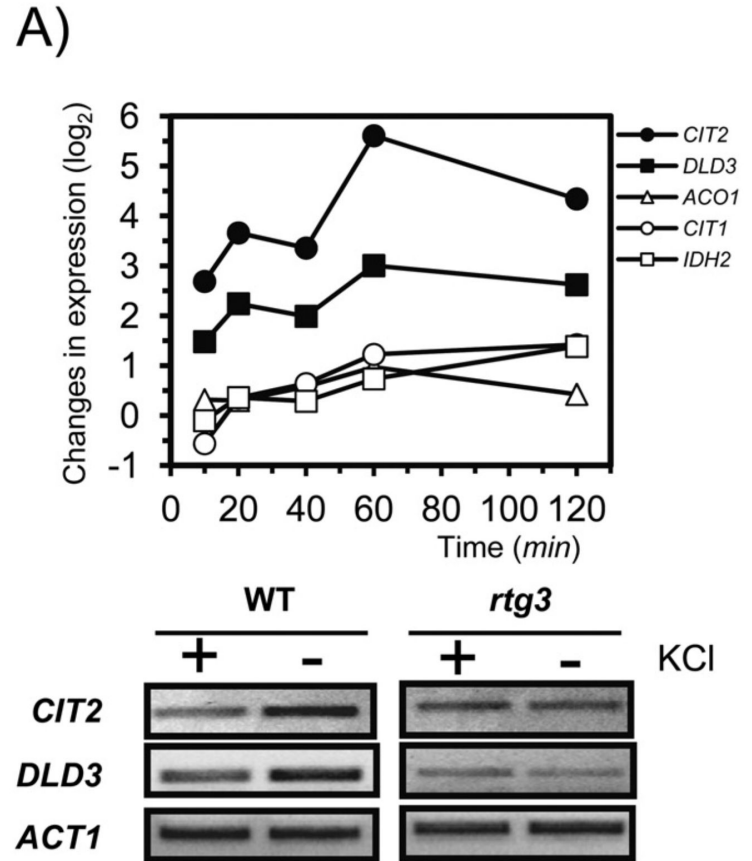


Fig. 5A: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 5B. K⁺ starvation leads to a strong increase in expression of CIT2 & DLD3 genes central to the retrograde response

- Mitochondrial morphology was normal, and not the cause of the retrograde response
- $\Delta fzo1$ mutant shows an abnormal mitochondrial morphology phenotype

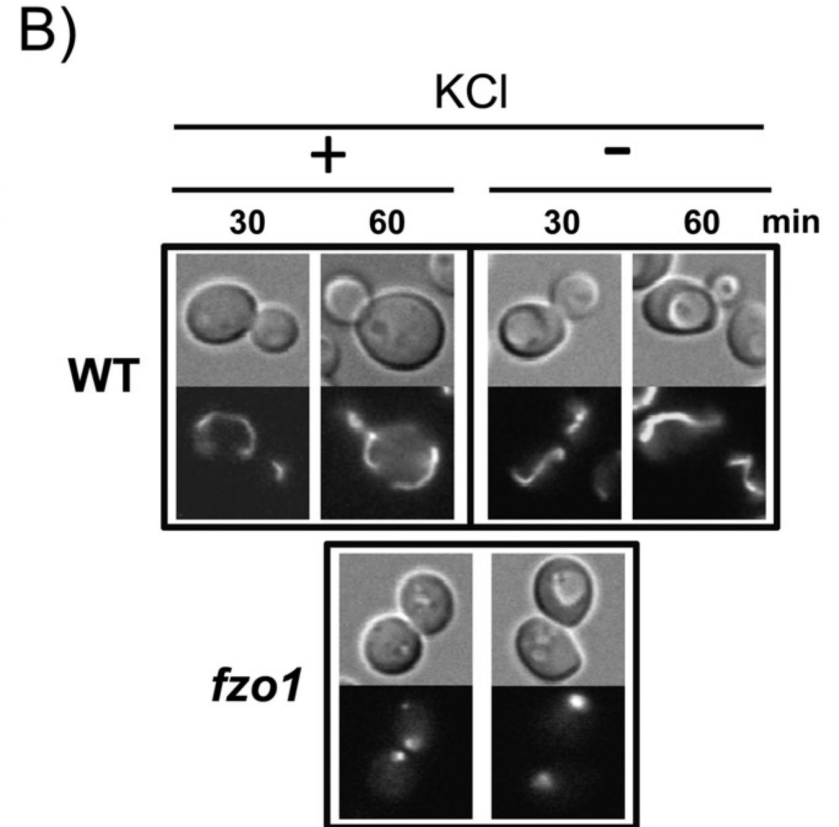


Figure 5C. Intracellular ammonium levels increase immediately after transfer to K^+ media

- Potassium starvation leads to an accumulation of ammonium in the cell, and may be one cause of the retrograde response
- Other studies have shown that retrograde gene response has been associated to an increase in intracellular ammonium content

C)

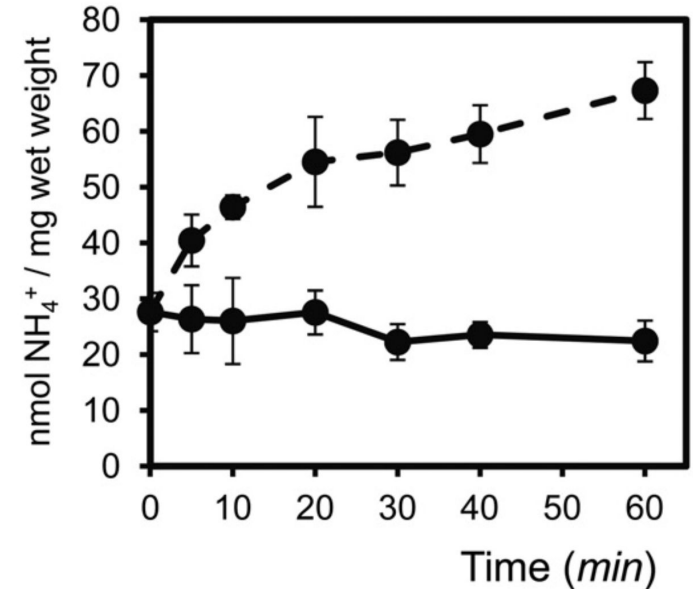


Figure 5D. Ammonium enters the cell utilizing the same transporters as potassium

- Cells without Trk1 do not show significant ammonium accumulation
- A Trk1 mutation that abolishes K^+ cation transport also shows significant decrease in ammonium accumulation

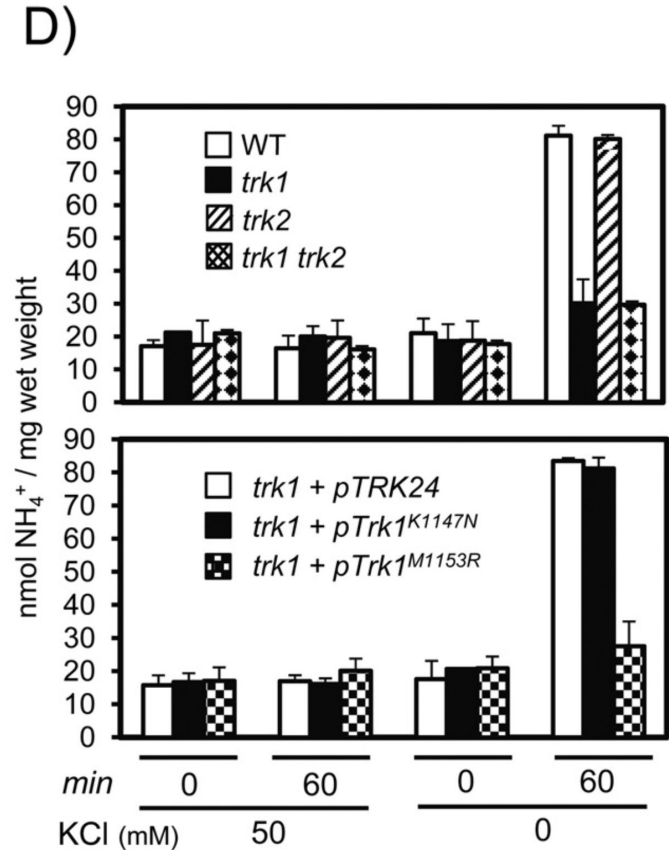


Fig. 5D: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 6. Expression of genes controlling cyclins exhibit a varied response to K⁺ starvation

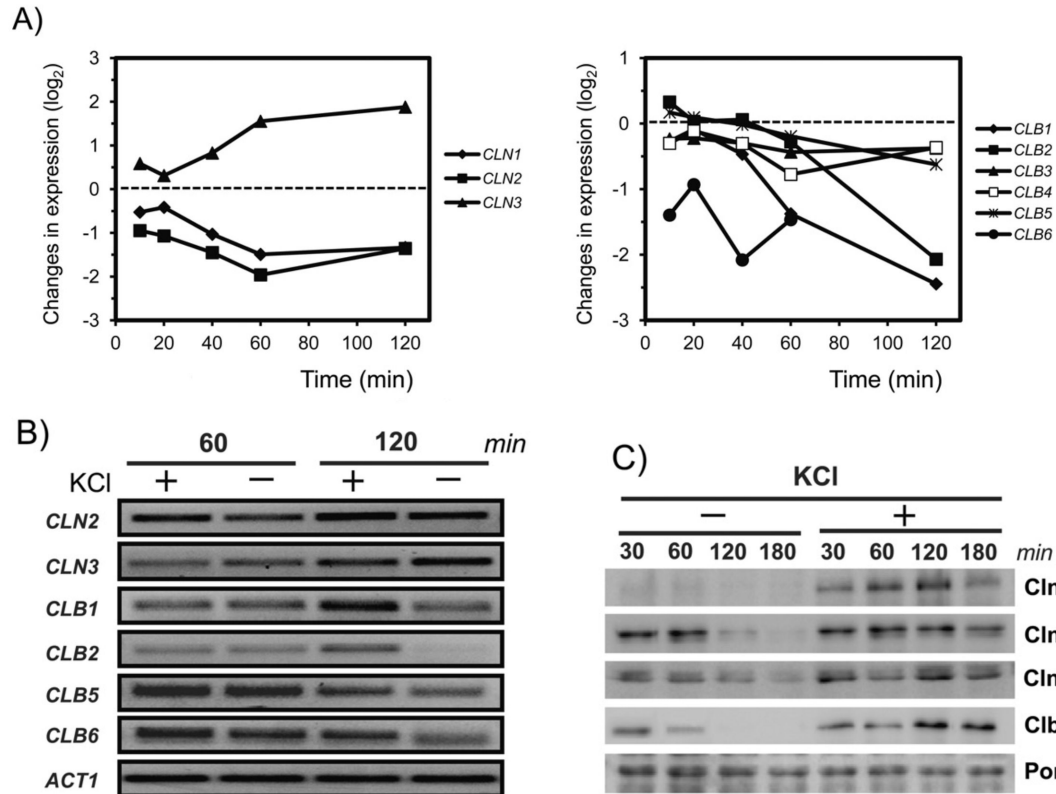


Fig. 6: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 7A: Lack of potassium in yeast destabilizes the septin ring

- Bud Neck and Septin Ring Formation DNA Expression Levels
- all three genes (GIN4, KCC4, HSL1) repressed
- mRNA levels for BUD4 also downregulated
- Microarray data indicated no relative change for septin genes

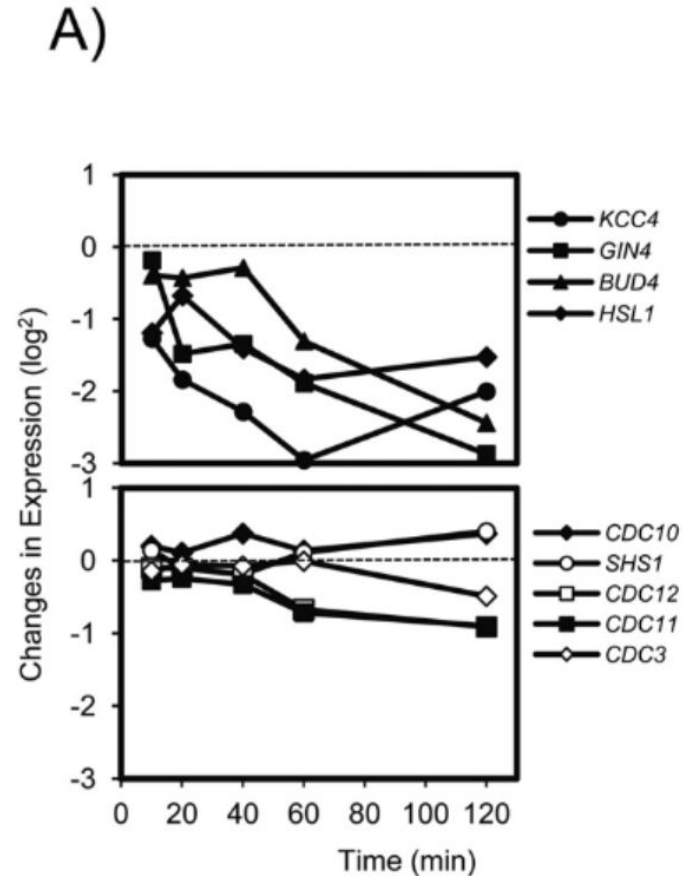


Figure 7B: CDC11-GFP localization is affected during K⁺ starvation

- one component of septin ring
 - after 30 min: appears distorted
 - after 60 min: no longer detectable

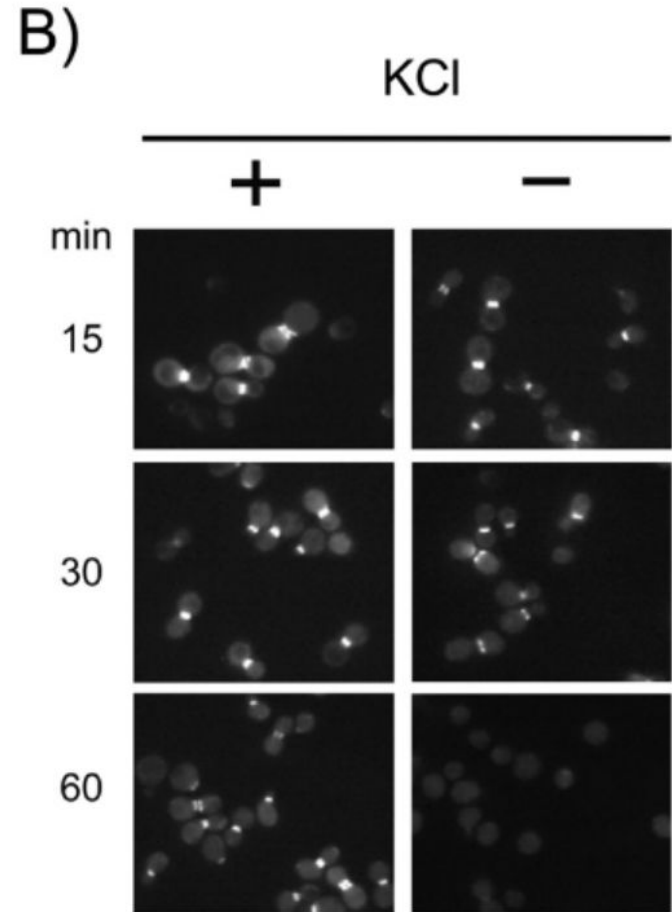


Figure 7C: CDC11 protein levels are constant during starvation

- Amounts of the septin were evaluated:
 - immunoblot in extracts of cells grown in (+) or (-) potassium
- Cdc11 remains constant in both fractions

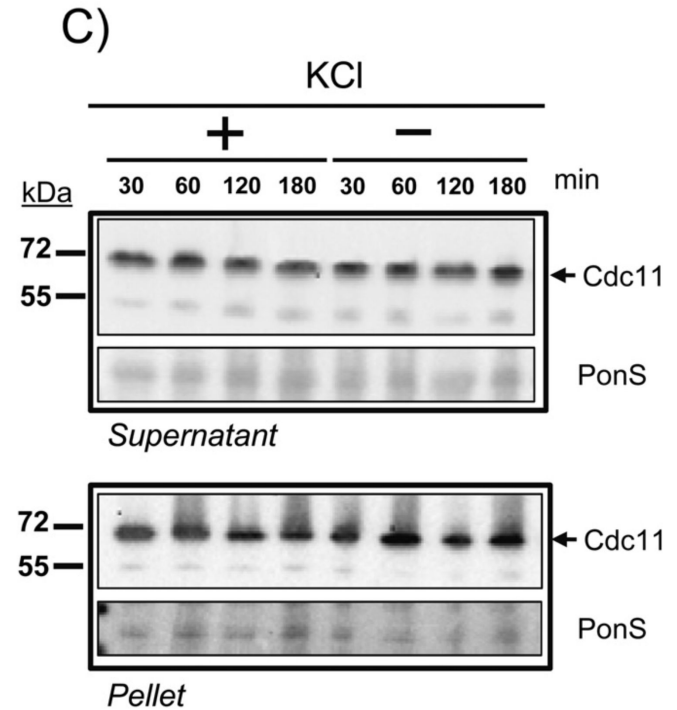
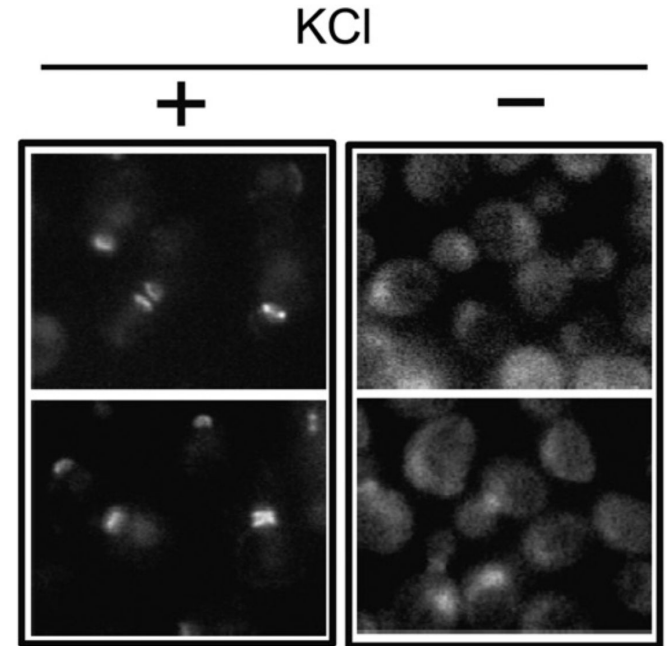


Figure 7D: CDC11 not properly localized in cells grown 60 min

- Immunolocalization experiment using anti-CDC11 antibodies
- mislocalization of the CDC11 septin

D)



Yeast Cells Modified Gene Expression in Response to Potassium Deprivation

- Major alterations in transcriptional profile
 - resulting induced or repressed at least at one time point
- Strong induction of most genes
 - sulfur utilization
 - Met/Cys import
 - biosynthesis
- Change in cells occur during retention of more than necessary potassium
- Strong increase in expression of genes related to trehalose metabolism
 - biosynthetic and degradative pathways
- Ammonium influx mediated by Trk1 potassium transporter
 - ability to transport potassium

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Potassium Starvation Changes Gene Expression

- Changes in gene expression impacted by potassium starvation
 - Sulfur metabolism and ammonium accumulation
 - Decrease in ribosome biogenesis and translation
- Uncovers more of role of potassium in the cell and its necessity for function
- Expands on specific pathways that are affected by deprivation
- Future Directions
 - Other cations and their effect on cellular function and gene expression

The Article Is Formatted Well But Has Its Limitations

- Elaborate on introduction
- Many figures to corroborate with results and findings
- Major concepts and ideas could have been introduced better and clearer
- Confusion with understanding the multitude of pathways

References

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Thank You
Dr. Dahlquist
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BIOL 367 Classmates