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

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

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.

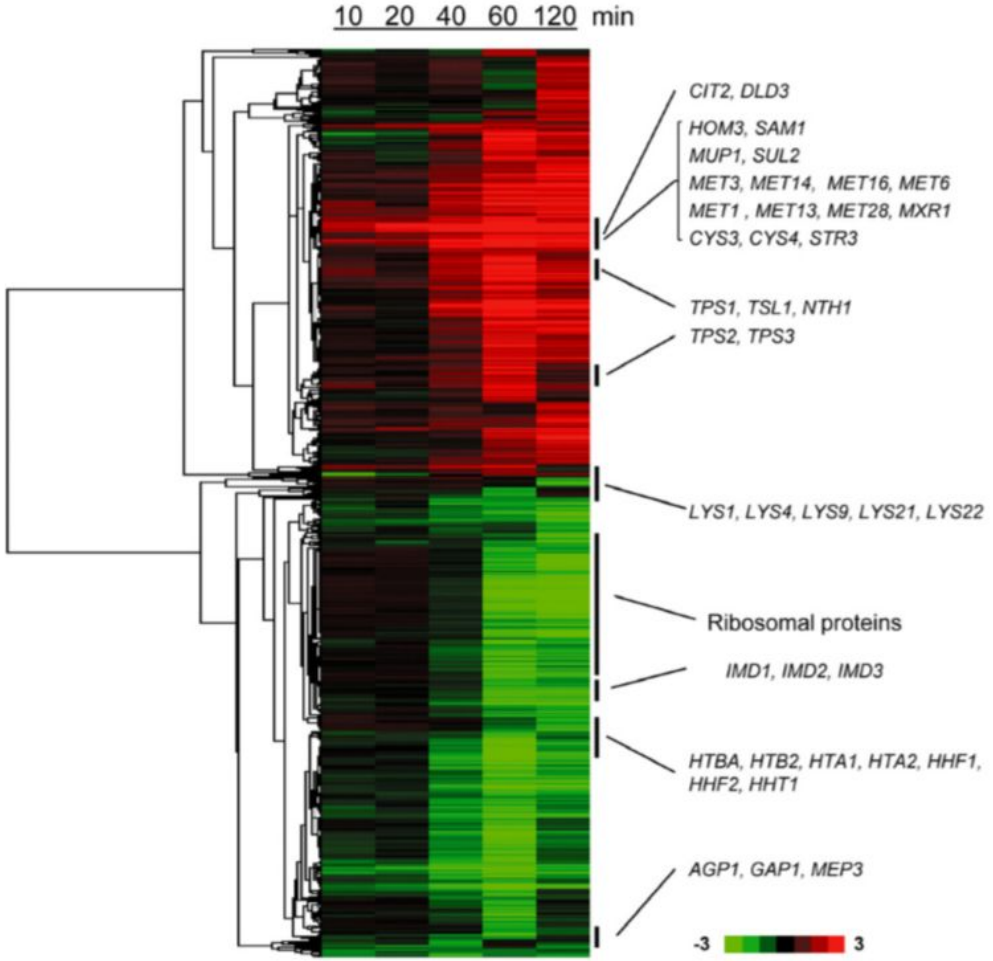

Fig. 1: Barretto et al. (2012) *Environmental microbiology*. 14:11

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.

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

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

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

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

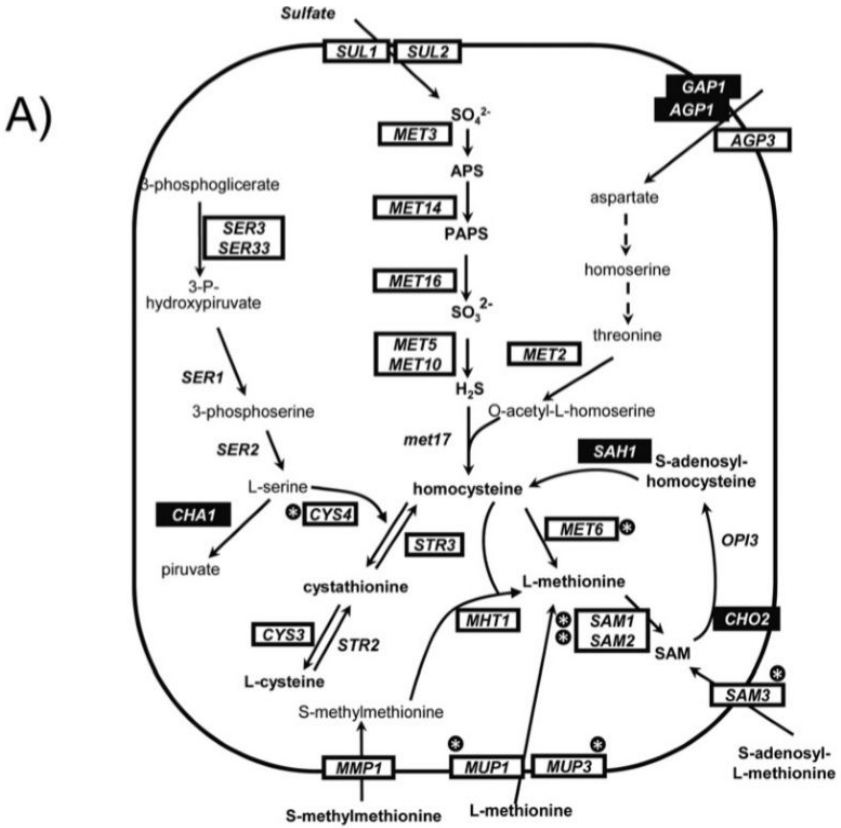

Fig. 2A: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

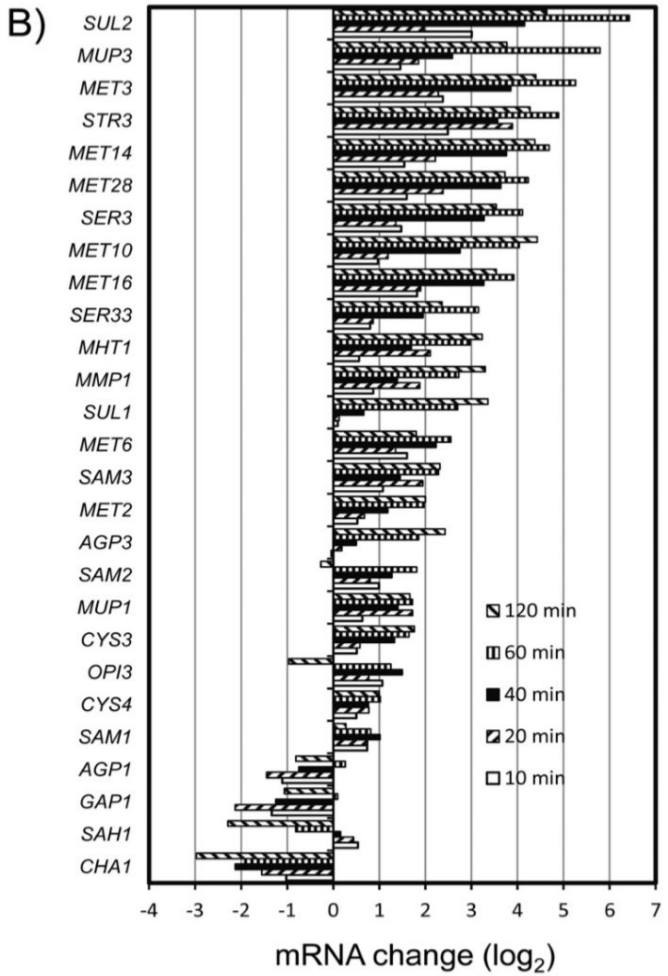

Fig. 2B: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

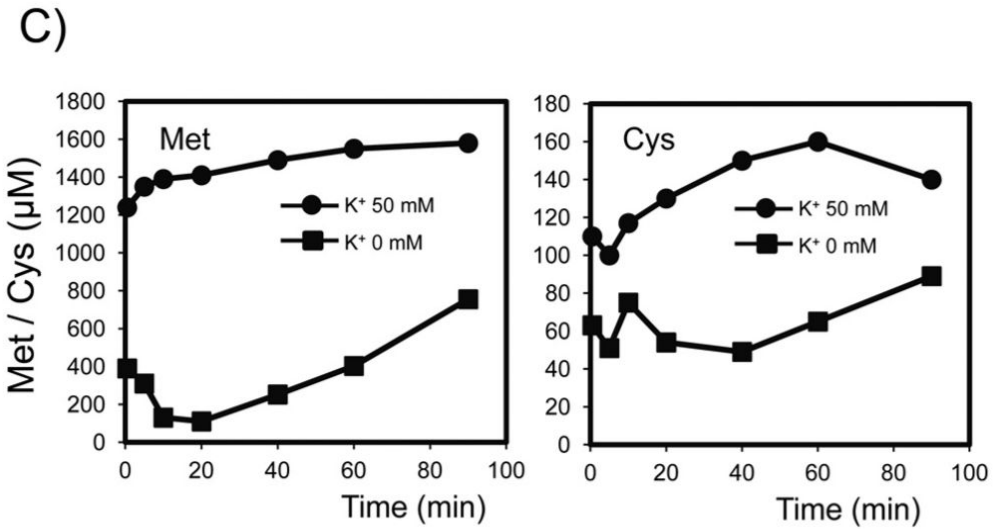

Fig. 2C: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

- After potassium starvation
- Measured through microarray experiments

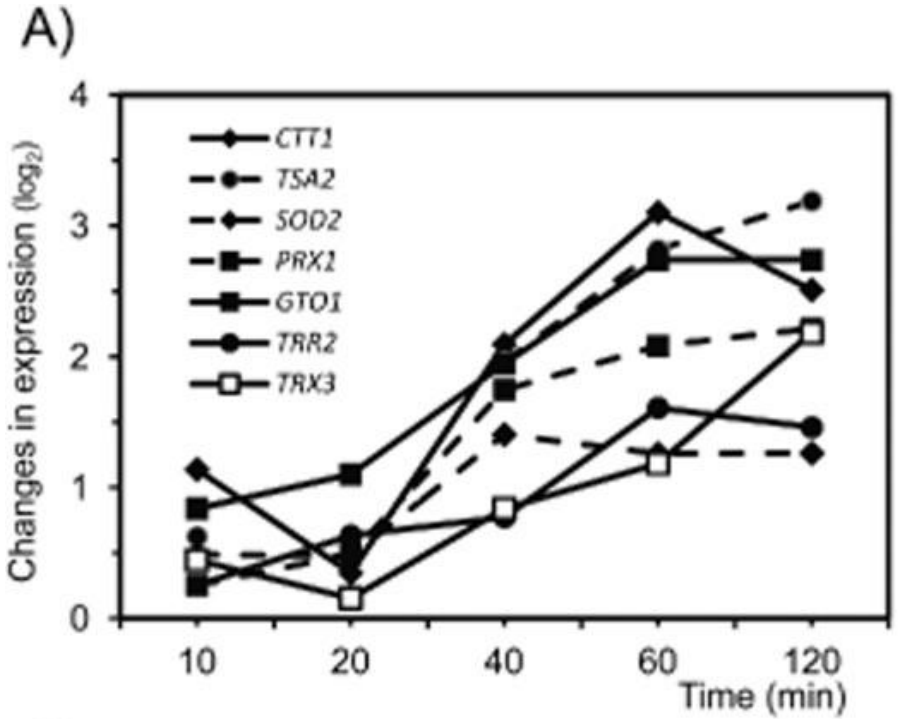

Fig. 3A: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

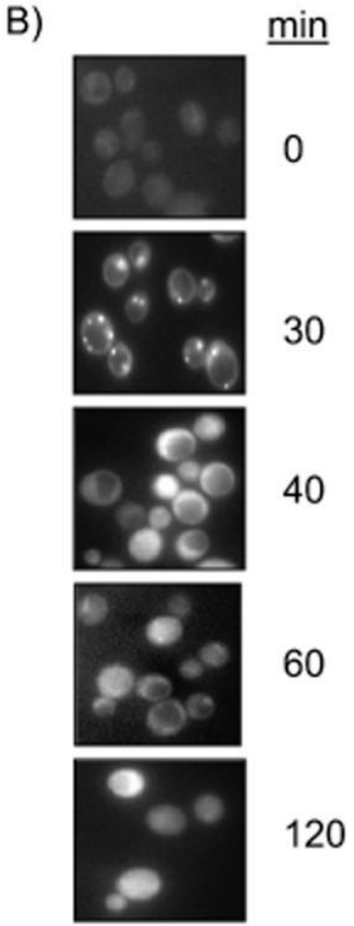

Fig. 3B: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

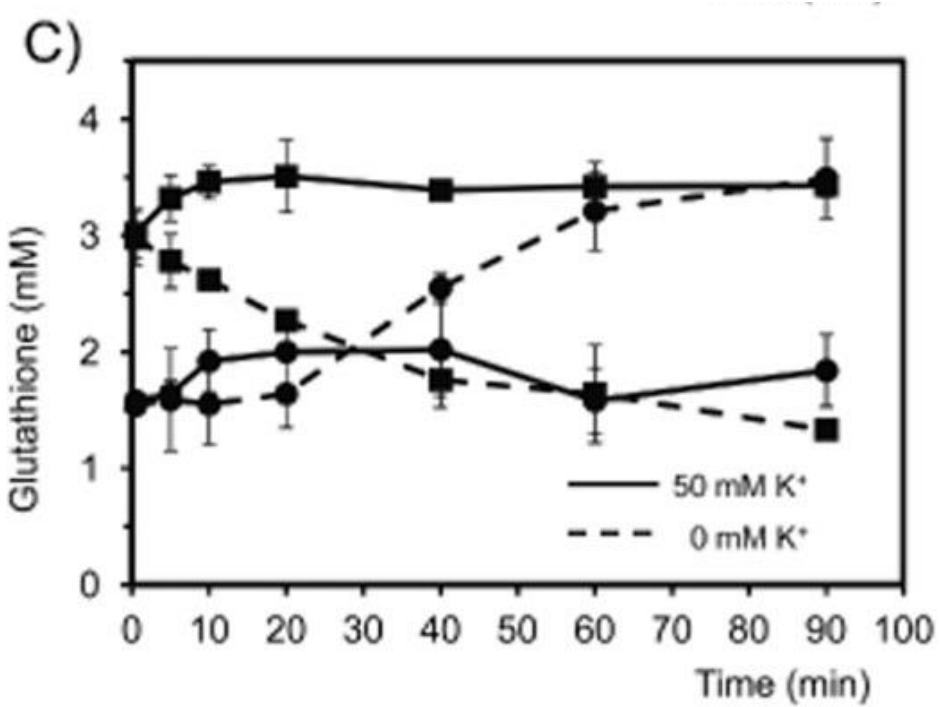

Fig. 3C: Barretto et al. (2012) *Environmental microbiology*. 14:11

Figure 4A: Methylglyoxal Increases with Trehalose Metabolism

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

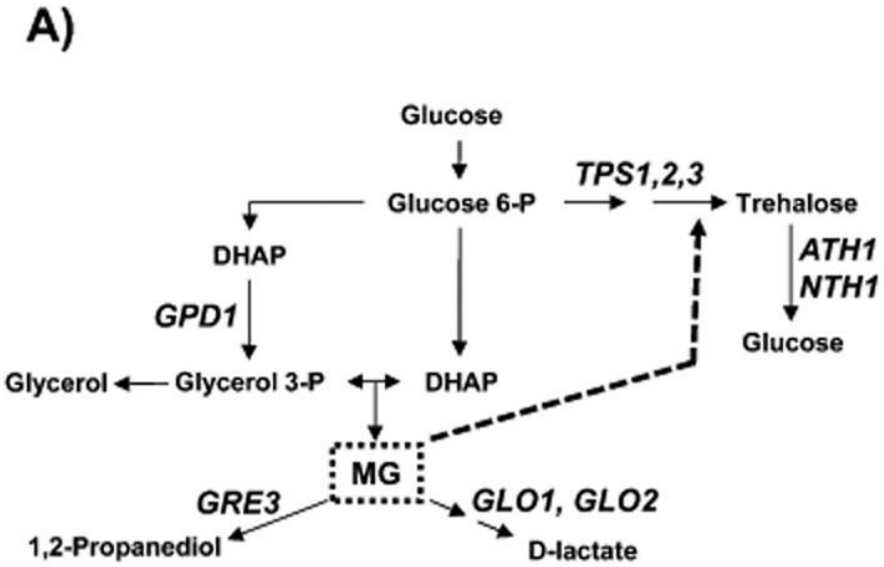

Fig. 4A: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

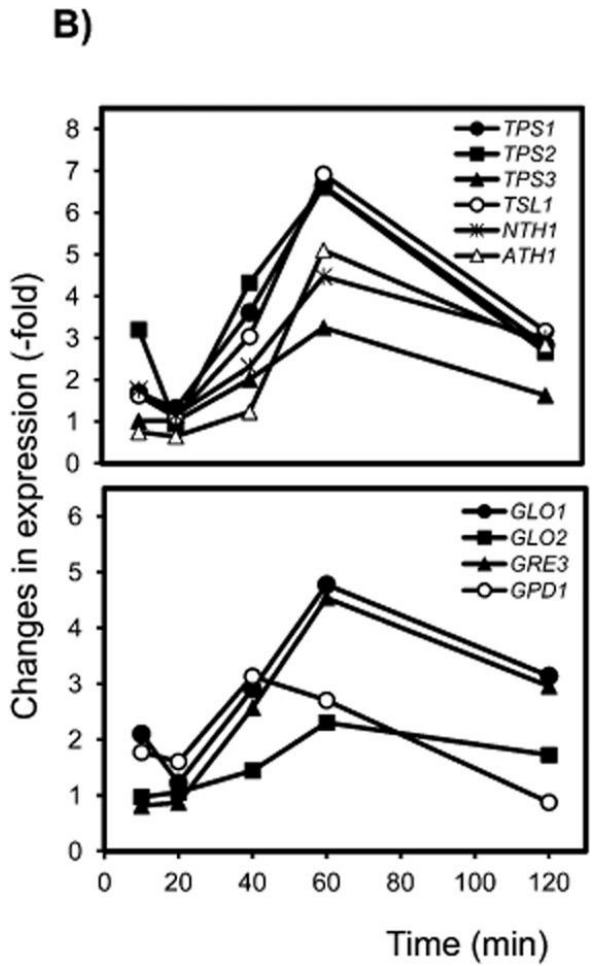

Fig. 4B: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

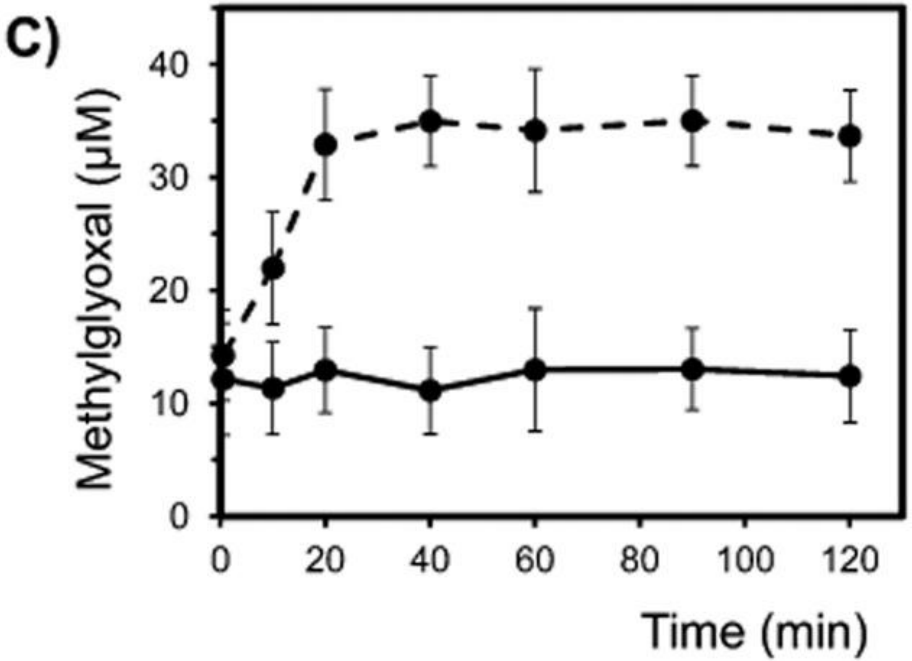

Fig. 4C: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

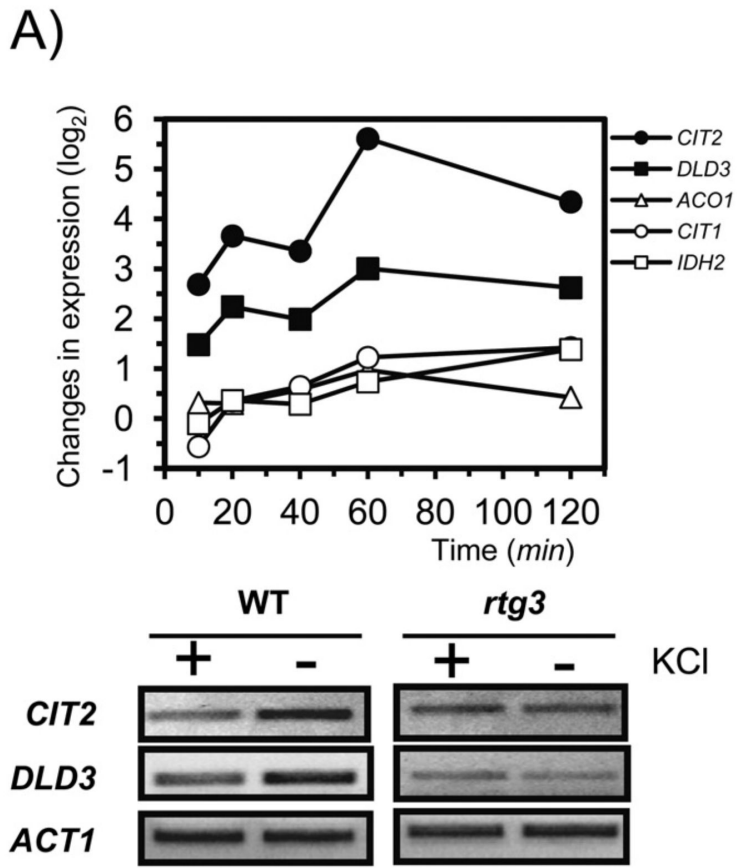

Fig. 5A: Barretto et al. (2012) *Environmental microbiology*. 14:11

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
- Δ*fzo1* mutant shows an abnormal mitochondrial morphology phenotype

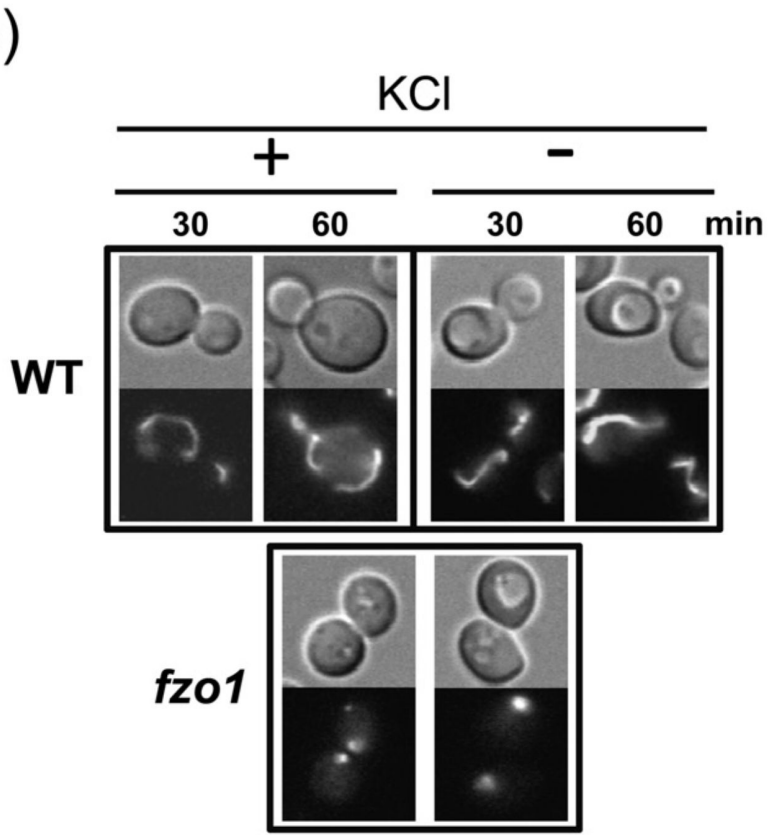

Fig. 5B: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

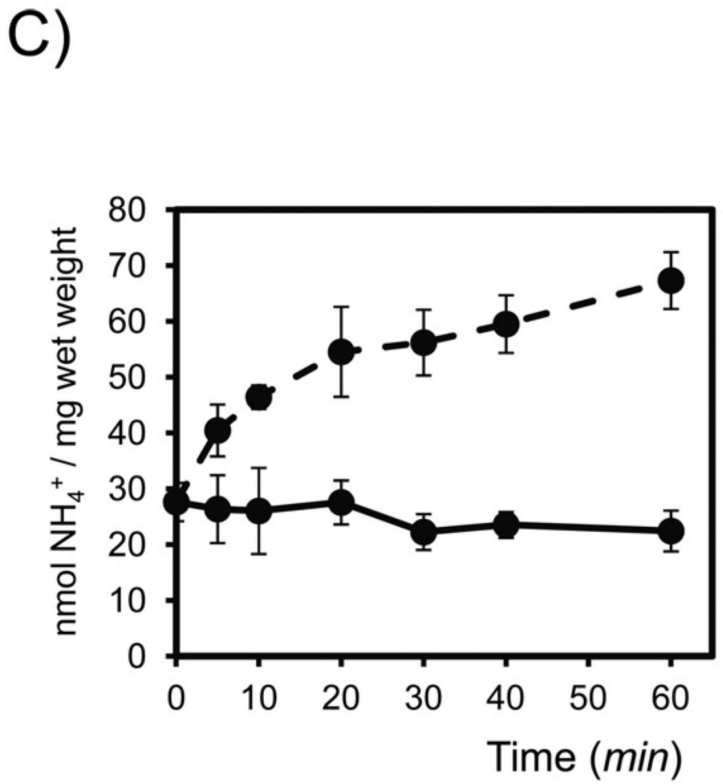

Fig. 5C: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

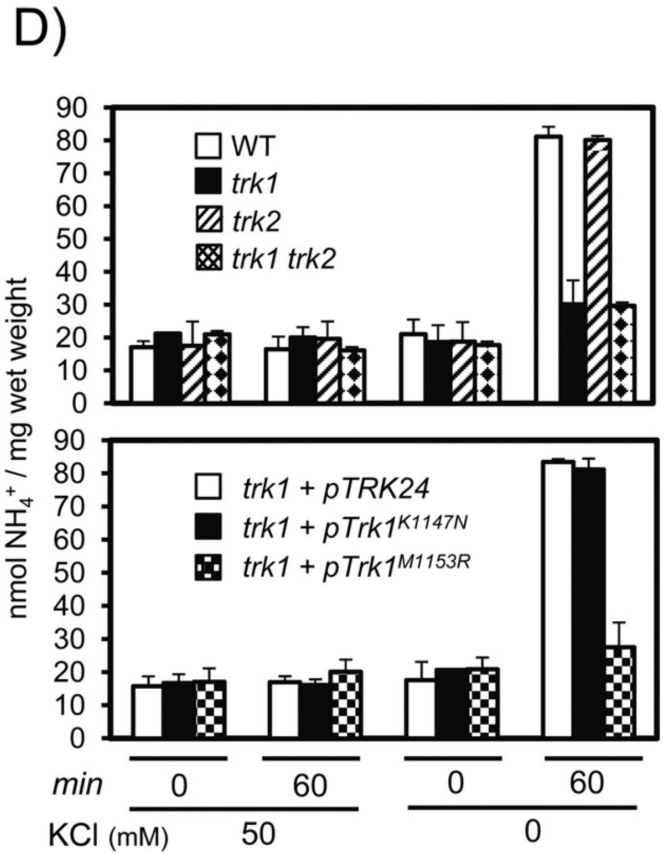

Fig. 5D: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

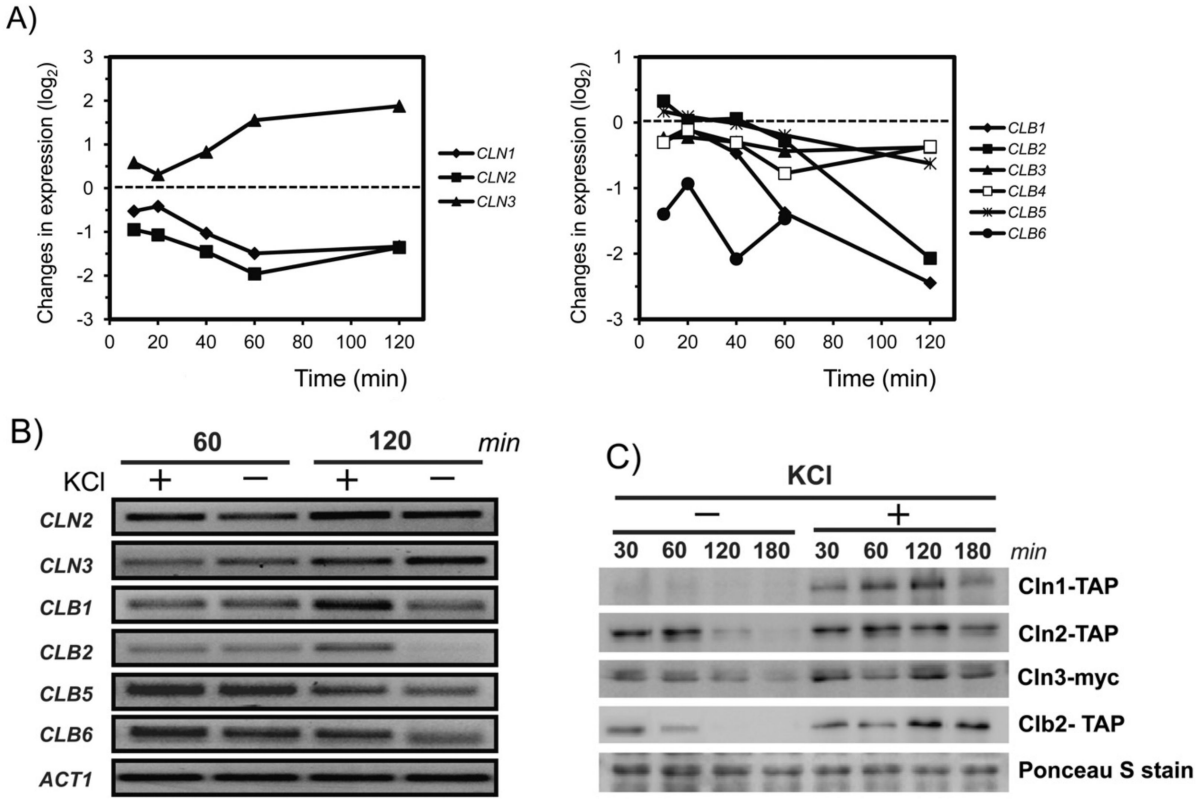

Fig. 6: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

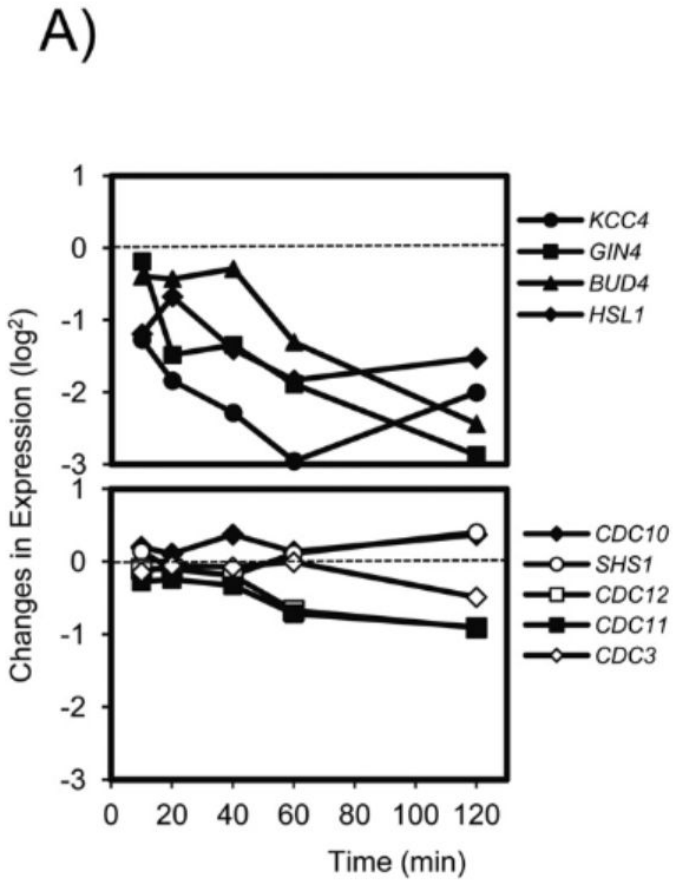

Fig. 7A: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

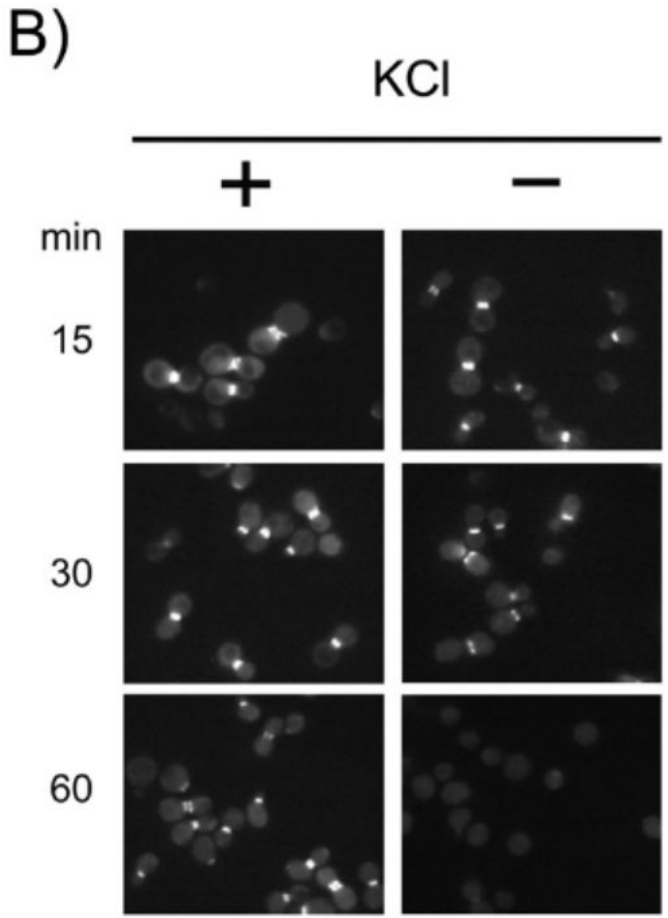

Fig. 7B: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

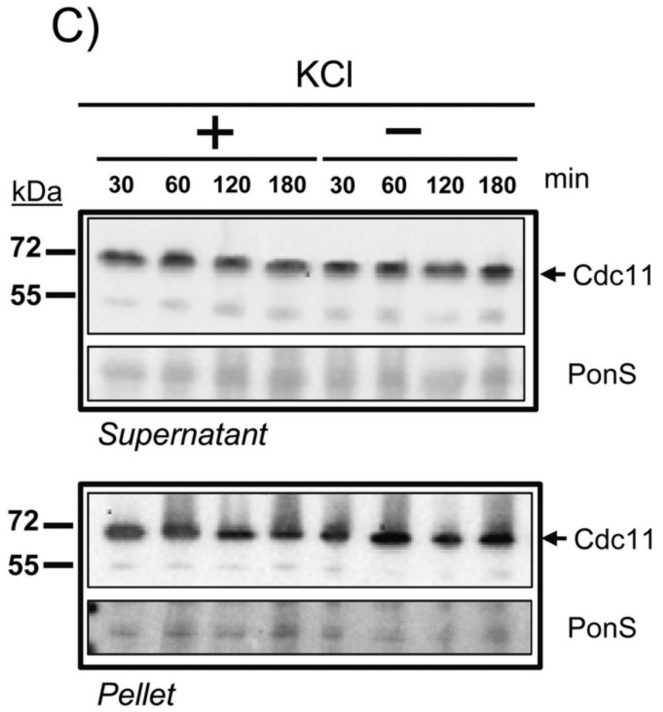

Fig. 7C: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

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

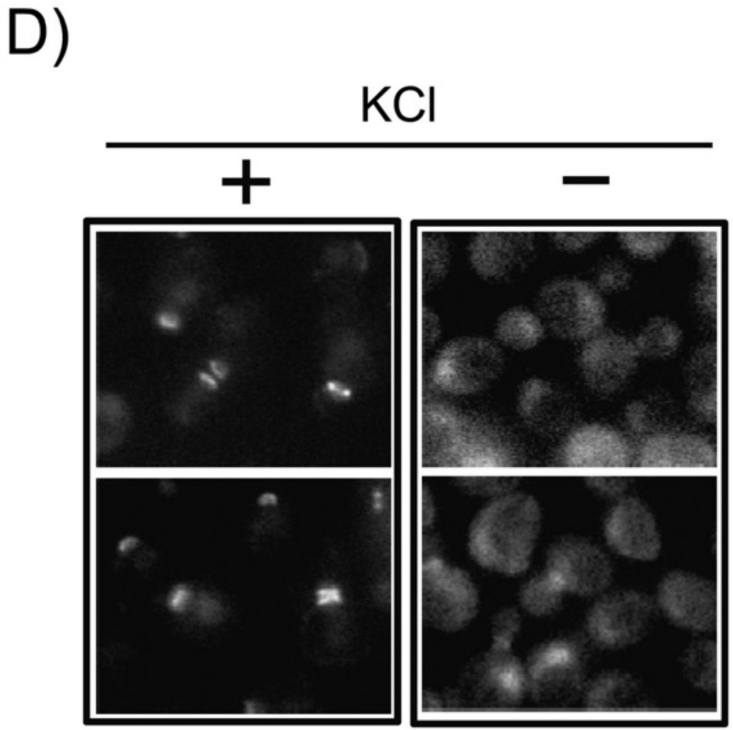

Fig. 7D: Barretto et al. (2012) *Environmental microbiology*. 14:11

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

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.

Hess, D. C., Lu, W., Rabinowitz, J. D., & Botstein, D. (2006). Ammonium toxicity and potassium limitation in yeast. PLoS biology, 4(11), e351.

Udensi, U. K., & Tchounwou, P. B. (2017). Potassium Homeostasis, Oxidative Stress, and Human Disease. International journal of clinical and experimental physiology, 4(3), 111–122. doi:10.4103/ijcep.ijcep_43_17

Thank You
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