Supplemental Materials

Molecular Biology of the Cell

Gibney et al.
Common and Divergent Features of Galactose-1-phosphate and Fructose-1-phosphate toxicity in yeast
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Supplemental Figure 1. Galactose titrations and IC₅₀ curves for galacose-1-phosphate toxicity

A - Growth curves for two biological replicates of the indicated strains in different basal media (YPD or SD) at different temperatures (30°C or 37°C) with increasing concentrations of galactose (0% to 2%). *Due to variation in starting inoculum for SD wild type and GAL1 at 30°C, the overlaid biological duplicates exhibit different lag times and were difficult to visualize; therefore each replicate is plotted alone.

B - Growth rates were calculated from two biological replicates (one is shown in panel A), then plotted against galactose concentrations for each replicate to generate IC₅₀ curves for each media condition and temperature indicated.
**S2A**

**wild type (DBY12000)**

- **SD - 30°C**
- **SD - 37°C**

**yrKHK (DBY12549)**

- **YPD - 30°C**
- **YPD - 37°C**

| Fructose Concentration (percent) | Color     |
|---------------------------------|-----------|
| 0.000                           | magenta   |
| 0.004                           | purple    |
| 0.008                           | blue      |
| 0.016                           | indigo    |
| 0.031                           | green     |
| 0.063                           | lime green|
| 0.125                           | yellow    |
| 0.250                           | orange    |
| 0.500                           | red       |
| 1.000                           | maroon    |
| 2.000                           | dark red  |
| 4.000                           | black     |
Supplemental Figure 2. Fructose titrations and IC$_{50}$ curves for fructose-1-phosphate toxicity

A - Growth curves for three biological replicates of the indicated strains in different basal media (YPD or SD) at different temperatures (30°C or 37°C) with increasing concentrations of fructose (0% to 4%)

B - Growth rates were calculated from three biological replicates, then plotted against fructose concentrations for each replicate to generate IC$_{50}$ curves for each media condition and temperature indicated.
Supplemental Figure 3. yrKHK-yEGFP is localized to the cytosol, though not excluded from the nucleus, and retains ability to cause Fru1P toxicity

A - yrKHK was tagged with a C-terminal yeast-codon-optimized EGFP (originally from pKT127, one of the GFP-tagging vectors from Kurt Thorn's lab). Cells were grown to early log phase in LFM before fixation and imaging. For cells in which fructose was added, cultures were again grown to log phase in LFM, then fructose was added to a final concentration of 2% for 1 hour before fixation and imaging. Imaging was performed with both MATa and MATα strains, obtained from independent transformations. For more details on sample preparation and imaging, see Materials and Methods.

B - Indicated strains were struck onto YNB medium with the indicated carbon sources, then incubated for 2 days at 30°C.
IC_{50} = 0.093
IC_{50} = 0.036
IC_{50} = 0.005
IC_{50} = 0.080
IC_{50} = 0.037
IC_{50} = 0.004

Yeast cells grown in YNB, YP, SD, YPD, SF, YPGal, and YPGal with increasing 2-deoxyglucose concentrations.

IC_{50} values:
- YNB: 0.093
- YP: 0.080
- SD: 0.036
- YPD: 0.037
- SF: 0.005
- YPGal: 0.004
Supplemental Figure 4. 2-deoxyglucose (2DG) as a model for sugar-phosphate toxicity

A - *Growth inhibition of 2-deoxyglucose is carbon-source dependent*. Growth curves for wild type (DBY12000) in different basal media (YP or YNB) at 30°C with indicated carbon source at 2% and an increasing concentration of 2-deoxyglucose. IC₅₀ values were calculated from this single replicate and are shown in red.

B - *Rapid growth inhibition without cell cycle arrest after addition of 2-deoxyglucose*. Cultures of wild type (DBY12000) cells were grown in YNB + 2% glucose media to mid-log phase before addition of 2-deoxyglucose to 1% (wt/vol). Cells were collected at the indicated time-points and examined for bud index (top panel), OD600 (middle panel), and plated onto YPD to measure survival based on colony forming units (lower panel). Each line represents one of three biological replicates.
S5A | 2-DG 0.2% | 2-DG 2% | GAL2 2% | yrKHK 0.2% | yrKHK 2% | GAL1/2 0.2% | GAL1/2 2%

fold change

> 8 fold down | > 8 fold up
Supplemental Figure 5. Gene expression response to sugar-phosphate accumulation

A - Entire gene expression data-set (a subset is shown in Figure 4). Included are 1 hour time-courses of DBY12000 treated with 2-deoxyglucose at 0.2% or 2% (“2-DG”), GAL2 overexpression strain treated with 2% galactose for 2 hours (“GAL2”), yrKHK strain treated with fructose at 0.2% or 2% for 2 hours (“yrKHK”), and GAL1/2 strain treated with galactose at 0.2% or 2% for 2 hours (“GAL1/2”). 2 hour time-courses include the following points: 0, 2.5, 5, 15, 30, 45, 60, and 120 minutes. Arrays were analyzed as described in methods (all floored to intensity of 350, missing data removed, zero-normalized for each time-course, then clustered using the Pearson uncentered metric with average linkage). 5,437 yeast genes are shown.

B - Heat map for microarrays shown in (A) before zero-normalization. Note that the experimental sample is labeled in red for each array, while the reference sample is labeled in green. Of the 38,059 expression values represented in this plot, only 1 gene is greater than 4-fold higher than reference (HSP26 in 2-DG 2%) and 8 genes are more than 4-fold lower than reference (all in GAL1/2 0.2%). Beyond a 2-fold threshold, 52 genes are higher than reference, while 99 genes are lower than reference. Most changes appear to be from arrays in which a different culture was used for the reference sample.
Supplemental Figure 6. elf1Δ suppression of fructose-1-phosphate toxicity can be complemented by adding ELF1 back on a plasmid (additionally, overexpression of ELF1 is mildly toxic, an effect more pronounced on galactose)

A - Indicated strains were grown overnight in SD liquid media before diluting to an OD_{600} = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose or fructose present at 2% when indicated). Plates were incubated for 2 days at 30°C before photographing.

B - Indicated strains were grown overnight in SD liquid media before diluting to an OD_{600} = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose or galactose present at 2% when indicated). Plates were incubated for 2 days at 30°C before photographing.
**S7A**

| Strain               | SD                | SD + fructose       | SD                | SD + fructose       |
|----------------------|-------------------|---------------------|-------------------|---------------------|
| **DBY12000**         | ![Image](image1)  | ![Image](image2)    | ![Image](image3)  | ![Image](image4)    |
| **yrKHK-C99R**       | ![Image](image5)  | ![Image](image6)    | ![Image](image7)  | ![Image](image8)    |
| **yrKHK**            | ![Image](image9)  | ![Image](image10)   | ![Image](image11) | ![Image](image12)   |
| **yrKHK/yrKHK-C99R** | ![Image](image13) | ![Image](image14)   | ![Image](image15) | ![Image](image16)   |

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**30°C**

**37°C**

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**S7B**

| Hs_KHK-A | Hs_KHK-C | yrKHK |
|----------|----------|-------|
| MEEKQILCVGLVVLDSVLVDKYKPEDSERCLSQRWQRGNNRSTVLSLGLAPCAF   | MEEKQILCVGLVVLDSVLVDKYKPEDSERCLSQRWQRGNNRSTVLSLGLAPCAF   | MEEKQILCVGLVVLDSVLVDKYKPEDSERCLSQRWQRGNNRSTVLSLGLAPCAF   |
| GSMAPGHVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham  | GSMAPGHVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham  | GSMAPGHVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham  |
| GSAHGVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham    | GSAHGVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham    | GSAHGVADFVLDLRSYVDRYTVFQQTGSVPITVIHAEASGSRTILY Durham    |
| SATDFEVLQTQFKWUERGRSVEQVMLQRIIDAHNTRQFPEQKIRVSVEEKPREELF | SATDFEVLQTQFKWUERGRSVEQVMLQRIIDAHNTRQFPEQKIRVSVEEKPREELF | SATDFEVLQTQFKWUERGRSVEQVMLQRIIDAHNTRQFPEQKIRVSVEEKPREELF |
| QLFYGVDFVVFSDVAKHLGFQSAEELRGLGVRKGVAVLCWAEAGADALPDKLL    | QLFYGVDFVVFSDVAKHLGFQSAEELRGLGVRKGVAVLCWAEAGADALPDKLL    | QLFYGVDFVVFSDVAKHLGFQSAEELRGLGVRKGVAVLCWAEAGADALPDKLL    |
| HSDAPPPREVQTLGAGTFNANSVISFLSQQRSGQEALELRFGCQQAVGKCCGLGFGDGV | HSDAPPPREVQTLGAGTFNANSVISFLSQQRSGQEALELRFGCQQAVGKCCGLGFGDGV | HSDAPPPREVQTLGAGTFNANSVISFLSQQRSGQEALELRFGCQQAVGKCCGLGFGDGV |

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Similar alignments with highlighted regions indicating sequence similarity.
Supplemental Figure 7. Identification of a dominant suppressor of fructose-1-phosphate toxicity by mutation of yrKHK at a conserved residue (C99R)

A - yrHK-C99R is dominant, though dominance is temperature sensitive; Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose and fructose present at 2%). Plates were incubated for 2 days at the indicated temperature before photographing.

B - yrHK-C99R mutation is present in the conserved KHK-C liver isoform; ClustalW2 alignment of Homo sapien (Hs) KHK isoforms A and C (C is the predominantly liver isoform) with yeast codon optimized rat liver ketohexokinase (yrHKH) used in this study. The region that varies in splice isoform C vs. A is highlighted in yellow. The mutated residue in the dominant suppressor (C99) is highlighted in red.

C - Suppressor mutation C99R is in a β-sheet present at the ketohexokinase dimerization interface. Blue arrows indicate the β-sheet where C99 is present in a crystal structure of the human KHKT isoform (structure is from 2009. Trinh CH et al. Acta Crystallographica Section D Biological Crystallography. D65: 201-211.)
Supplemental Figure 8. Overexpression of GCR1 and MOT3 restore fructose-only growth to yrKHK, but not galactose-only growth to GAL1/2.

The indicated strains were spotted onto the indicated media (from prepared 10-fold serial dilutions). Plates were incubated at 30°C for 3 days before photographing.
Supplemental Figure 9. Adding exogenous phosphate to the media does not repair growth inhibition resulting from sugar-phosphate accumulation

A - Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose present at 2%; galactose present at 2% when indicated). Plates were incubated for 2 days at 30°C before photographing. The number in parenthesis after the added potassium phosphate amount indicates the relative amount of potassium phosphate compared to SD alone.

B - Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose present at 2%; fructose present at 2% when indicated). Plates were incubated for 2 days at 30°C before photographing. The number in parenthesis after the added potassium phosphate amount indicates the relative amount of potassium phosphate compared to SD alone.
Supplemental Figure 10. Neither overexpression of MES1 (methionyl-tRNA synthetase) nor supplementation with methionine metabolites repairs galactose-1-phosphate toxicity

A and B - Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto the indicated media (glucose present at 2%; galactose present at indicated concentrations). Methionine was added to a final concentration of 1 g/L (standard minimal media formulations contain 20-85.6 mg/L, therefore this is 11-50X). S-adenosyl methionine (SAM) was added to a final concentration of 1 mM (other studies have used 0.1 - 0.5 mM to examine suppression of phenotypes by SAM). Plates were incubated for 2 days at 30°C before photographing.
|                  | p-aminobenzoic acid | calcium | biotin | folic acid | inositol | niacin | riboflavin | thiamine |
|------------------|----------------------|---------|--------|-----------|----------|--------|-----------|---------|
| - galactose      |                      |         |        |           |          |        |           |         |
| + galactose      |                      |         |        |           |          |        |           |         |

**S11B**

|                  | Wild type | GAL1 | GAL2 | GAL1/2 |
|------------------|-----------|------|------|--------|
| + 2% galactose   | ![Image](wild type.png) | ![Image](GAL1.png) | ![Image](GAL2.png) | ![Image](GAL1/2.png) |
| + 2% galactose + 2% ribose | ![Image](wild type.png) | ![Image](GAL1.png) | ![Image](GAL2.png) | ![Image](GAL1/2.png) |
| + 2% galactose + 2% DHA | ![Image](wild type.png) | ![Image](GAL1.png) | ![Image](GAL2.png) | ![Image](GAL1/2.png) |

**S11C**

|                  | SD | SD + 2% galactose | SD + 2% fructose |
|------------------|----|------------------|-----------------|
| Wild type        | ![Image](wild type.png) | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) |
| GAL1/2           | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) |
| Wild type        | ![Image](wild type.png) | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) |
| yrKHK            | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) | ![Image](GAL1/2.png) |
Supplemental Figure 11. Various supplements do not suppress sugar-phosphate toxicity

A - Vitamin supplementation does not remediate galactose-phosphate toxicity; GAL1/2 strain was grown overnight in SD before dilution into SD + 1% galactose with vitamin supplements and grown in a plate reader (Biotek Synergy H1) for 24 hours. Shown are the traces produced by the plate reader software. Vitamin supplements are added at 60X the concentration found in the standard yeast nitrogen base (YNB) formulation.

B - Neither ribose nor dihydroxyacetone (DHA) supplementation remediate galactose-phosphate toxicity; Indicated strains were grown overnight in YPD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto YPD with the indicated supplements. Plates were incubated for 2 days at 30°C before photographing.

C - Hypoxic growth does not remediate galactose- or fructose-phosphate toxicity; Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto SD with the indicated supplements. Plates were incubated for 4 days at 30°C in an anoxic chamber before photographing (colorimetric test strip in the chamber indicated oxygen levels below the limit of measurement).

D - Osmotic stabilization of cells with 1M sorbitol does not remediate galactose-phosphate toxicity; Indicated strains were grown overnight in SD liquid media before diluting to an OD$_{600}$ = 1.0. Cell suspensions were serially diluted using 1:10 steps, then spotted onto SD with the indicated supplements. Plates were incubated for 2 days at 30°C before photographing.
**Supplemental Figure 12. Comparison of sugar-phosphate suppressors from previous literature.**
The indicated strains were spotted onto the indicated media (from prepared 10-fold serial dilutions). Plates were incubated at 30°C for 2-3 days as indicated before photographing.
Supplemental Figure 13. Detoxification of accumulated sugar-phosphates prolongs first doubling after removal of toxic sugar. Indicated strains (all diploids) were grown to mid-log phase before treatment with relevant toxic sugar for 10 hours (2% of 2DG, fructose, or galactose, respectively). Cells were then pelleted and washed three times in YNB + 2% glucose before inoculation into YNB + 2% glucose medium in the Biotek Synergy HT plate reader (at an OD600 value of roughly 0.18 - within the linear range of detection for the instrument). Doubling times were calculated for strains that were not exposed to toxic sugar (blue bars). After release from toxic sugar, first doubling (red bars) and second doubling (green bar) times were measured, demonstrating that the first doubling is extended, presumably to detoxify the intracellular sugar-phosphates. Error bars represent standard deviation of three biological replicates.
DOWNLOADABLE DATA INFORMATION

Common and Divergent Features of Galactose-1-phosphate and Fructose-1-phosphate Toxicity in Yeast
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File: “Gibney et al - Comparison Gene Lists.txt”
- Contains gene lists used for microarray comparisons shown in Figure 4
- Gasch 2000 columns are from Gasch et al. 2000. MBC. 11: 4241-4257.
- Brauer 2008 columns are from Brauer et al. 2008. MBC. 19: 352-367.

File: “Gibney et al - Sugar Phosphate Arrays.txt”
- Contains data from sugar-phosphate perturbation microarrays performed as described in Materials and Methods
- Values in the table represent log2-transformed ratio of experimental signal compared to reference signal (the reference RNA used was the time-zero point for each, independent time-course); data was also zero-normalized before clustering shown in Figure 4
- Column labels indicate the following (where XX indicates minutes, though 2.5 minutes is represented as 2.5 in the table):
  - SD+lowDG_tXX: treatment of wild type cells with 0.2% 2-deoxyglucose
  - SD+highDG_tXX: treatment of wild type cells with 2% 2-deoxyglucose
  - GAL2OE+highGal_tXX: treatment of GAL2 cells with 2% galactose
  - yrKHK+lowFru_tXX: treatment of yrKHK cells with 0.2% fructose
  - yrKHK+highFru_tXX: treatment of yrKHK cells with 2% fructose
  - GAL1/2OE+lowGal_tXX: treatment of GAL1/2 cells with 0.2% galactose
  - GAL1/2OE+highGal_tXX: treatment of GAL1/2 cells with 2% galactose

File “Gibney et al - yrKHK Recessive Suppressor Array.txt”
- Contains data from a single microarray: experimental RNA was derived from the original, recessive yrKHK suppressor (described in Materials and Methods); reference RNA was derived from wild type cells; both cultures were grown in minimal medium (SD) to log phase before collection of cells and RNA extraction
- Values in the table represent the log2-transformed ratio of experimental signal to reference signal (the column heading, “yrKHK_REC,” indicates the recessive suppressor)

File “Gibney et al - Sugar Phosphate Metabolomics.txt”
- Contains data from metabolomics analysis described in Materials and Methods (Instruments QE1 and QE2 indicate methods 1 and 2, respectively)
- Values in the table represent the peak height for each indicated compound; before clustering and visualization (shown in Figure 5), data were floored to a value of 5000, zero-normalized, and log2-transformed.
- Column labels indicate the following (where XX indicates minutes):
  - GAL(rep1)_XX: treatment of GAL1/2 grown in SD with 1% galactose, biological replicate 1
  - GAL(rep2)_XX: treatment of GAL1/2 grown in SD with 1% galactose, biological replicate 2
  - FRU(rep1)_XX: treatment of yrKHK grown in SD with 1% fructose, biological replicate 1
  - FRU(rep2)_XX: treatment of yrKHK grown in SD with 1% fructose, biological replicate 2