Yeast genomics reveals spill chemical impact on cellular processes

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Abstract

In 2014, the coal cleaning chemical 4-methylcyclohexanemethanol (MCHM) spilled into the water supply for 300,000 West Virginians. Initial toxicology tests showed relatively mild results, but the underlying effects on cellular biology were underexplored. The existing resources of the yeast genetics community create a model system for extensive exploration of the effects of such chemicals. Treated wild type cells grew poorly, but viability experiments showed only a small decrease, leading us to believe yeast cells arrested in response to MCHM. Cell cycle analysis via flow cytometry of asynchronous cells revealed a complete absence of cells in S phase within thirty minutes of treatment. Cells accumulated in G1 over a six hour time course. A genetic screen of all haploid knockout mutants from the BY4742 collection revealed 330 genes required for optimal growth in MCHM. According to GO term analysis, these knockout strain genes belong to three major cell processes: mitochondrial gene expression/translation, the vacuolar ATPase, and aromatic amino acid biosynthesis. The accompanying RNA-seq dataset for MCHM treated cells showed an increase in expression of pleiotropic drug response genes and amino acid biosynthetic genes, and a decrease in ribosome biosynthesis. Analysis of the two genomic datasets in combination with observed cell cycle arrest revealed that the environmental stress response (ESR) was activated upon treatment. Both datasets agreed that the aromatic amino acid genes *ARO1*, *ARO3*, and four of the five *TRP* genes, were required for response to MCHM. This implicated nutrient deprivation as the cause of the ESR activation. Yeast were grown in rich media, so the source of nutrient deprivation was elusive. We hypothesized that the rich media may be lacking in the necessary amounts of aromatic amino acids, so rich media was further supplemented with excess tryptophan, tyrosine, and phenylalanine to compensate. However, excess supplementation did not improve growth on MCHM. Previous metabolomics analysis showed that amino acid levels increased on MCHM, so the source of nutrient deprivation signal and the function of these pathways in response to MCHM is still unclear. The combined datasets also implicated the importance of mitochondria and the vacuole in MCHM treated yeast. Previous work showed that petite yeast lacking mitochondria were sensitive to MCHM. We hypothesized that these observations were all related to reactive oxygen species (ROS) homeostasis. Flow cytometry with ROS-reactive dye revealed an increase of ROS in treated cells. A comet assay detected DNA damage as well with MCHM treatment. MCHM appears to cause cell cycle arrest and DNA damage through the accumulation of ROS. We propose that arrested cells survive through implementation of robust environmental stress responses, but there are unknown roles of homeostatic ROS and nutrient biosynthetic pathways to be further explored.

Cellular Arrest Phenotype

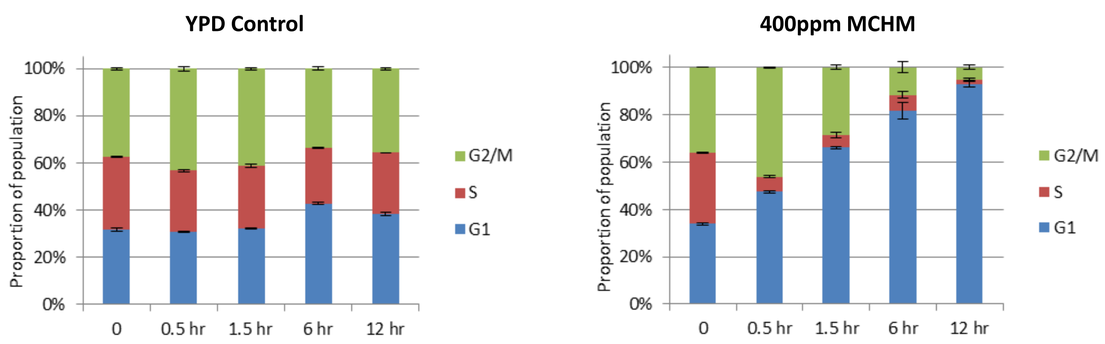


Figure 1 Cell Cycle Analysis of MCHM Treated Yeast. Initial tests of cell viability revealed that yeast cells were not dying from acute MCHM treatment (data not shown). Therefore, cell cycle analysis was employed to determine if cells were arresting instead. Analysis confirmed that yeast are arresting in G1. Cell cycle arrest is a hallmark of initial stages of the environmental stress response, supporting the hypothesis that it is activated upon exposure to MCHM.

Genetic Screen Method

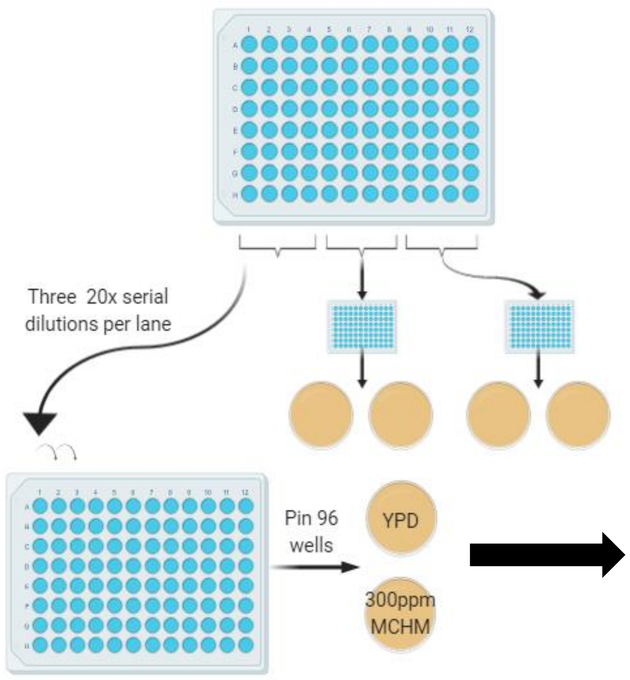
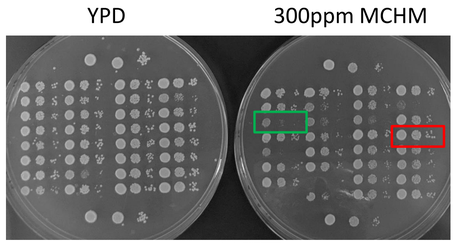


Figure 2 Genetic Screen Experimental Design. The approximately sixty 96-well plates containing the frozen BY4742 yeast knockout collection (each haploid strain contains one of the ~4500 non-essential genes of the yeast genome knocked out) were grown up for 2 days to saturation. Saturated cultures were serially diluted 20-fold to create 3 spots of growth assay concentrations for each strain. These were then plated and scored as below. The pictured plate shows a green box consistent with a "hit" in the screen and red box consistent with a strain that is not affected by MCHM exposure.



Genomic Datasets and Follow-up Experiments

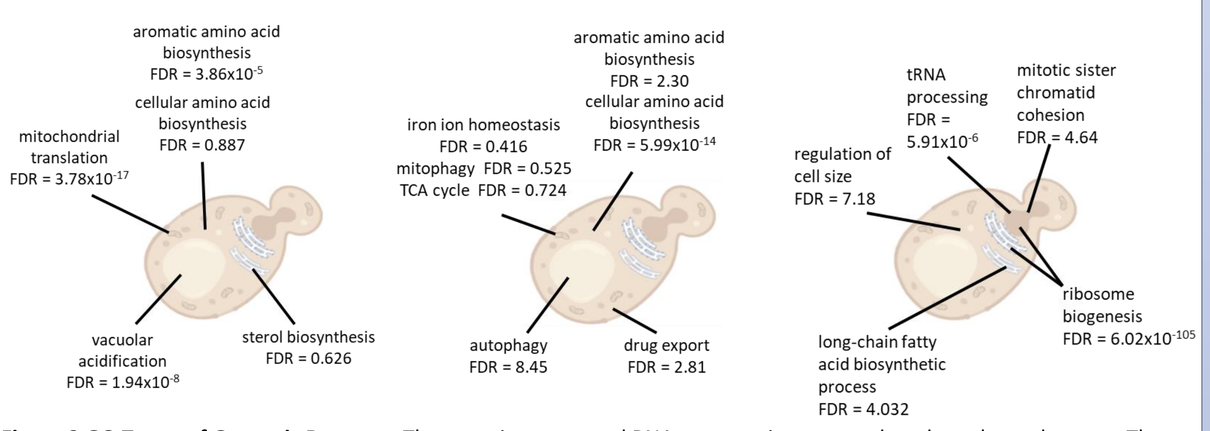


Figure 3 GO Terms of Genomic Datasets. The genetic screen and RNAseq experiments produced two large datasets. The screen of knockout mutants showed 330 genes required for tolerance of MCHM exposure on solid growth media. The GO Terms for this dataset pointed to the vacuolar ATPase, and presumably its role in ion, pH, and metal homeostasis. The mitochondrial translation genes in this case are key to proper mitochondrial function. Previous work showed petite yeast cells without functional mitochondria are sensitive to MCHM. Aromatic amino acid biosynthesis genes are required, despite the fact that the yeast were grown on YPD, rich media supplying all required nutrients the cell needs. The upregulated genes of the RNAseq dataset share pathways with the genetic screen, including metal ion homeostasis, mitochondrial function, and amino acid biosynthesis. The upregulated and downregulated genes combined point to a key hypothesis that MCHM is activating yeast's environmental stress response programming (downregulate ribosomes, upregulate autophagy and other stress responses such as drug exporters). FDR is reported for multitesting correction.

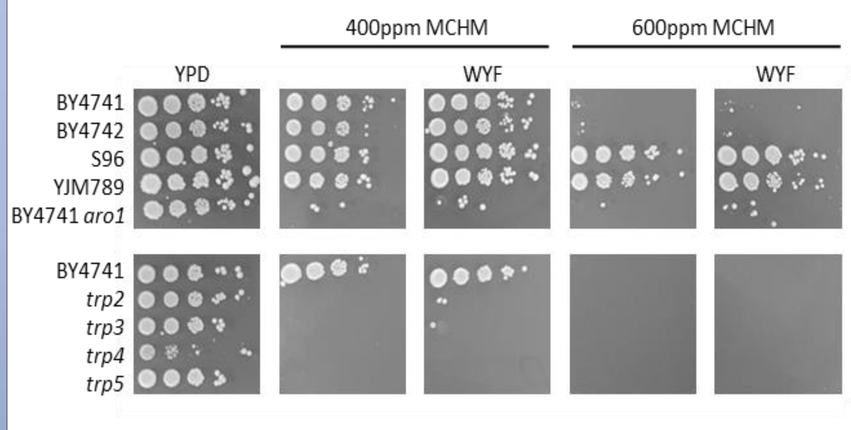


Figure 4 Aromatic Amino Acid Biosynthesis genes phenotyping. The overlap of the knockout screen and RNAseq included the *trp* genes and several *aro* genes. These genes are required for production of tryptophan and all aromatic amino acids respectively. Supplementation with the 3 aromatic amino acids (WYF) was unable to rescue MCHM growth phenotypes of the knockouts, so it is unclear what role these genes play in MCHM tolerance if not due to aromatic amino acid availability.

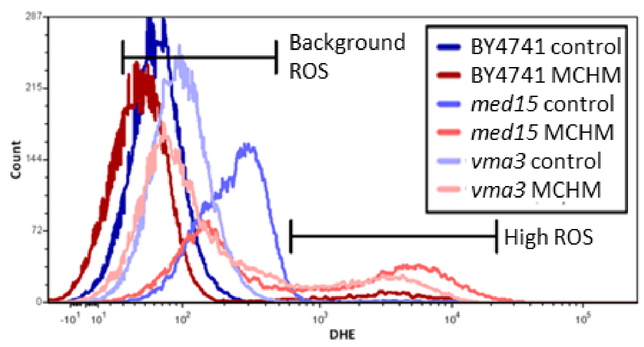


Figure 5 Flow Cytometry Assay for ROS. Genomic data showed mitochondrial and vacuolar homeostatic processes (Fig 3) and upregulation of genes associated with reactive oxygen species stress (data not shown). Flow cytometry of cells treated with MCHM and dyed with the ROS reactive dye DHE indicate that a subpopulation of yeast have very high ROS. *med15* and *vma3* are mutants for genes required for various stress responses. These mutants show even higher ROS production with MCHM treatment, revealing the importance of robust homeostatic process for MCHM tolerance.

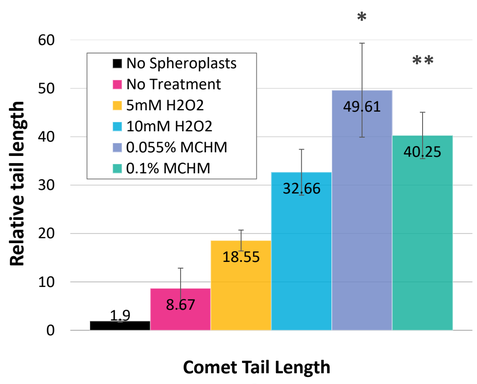
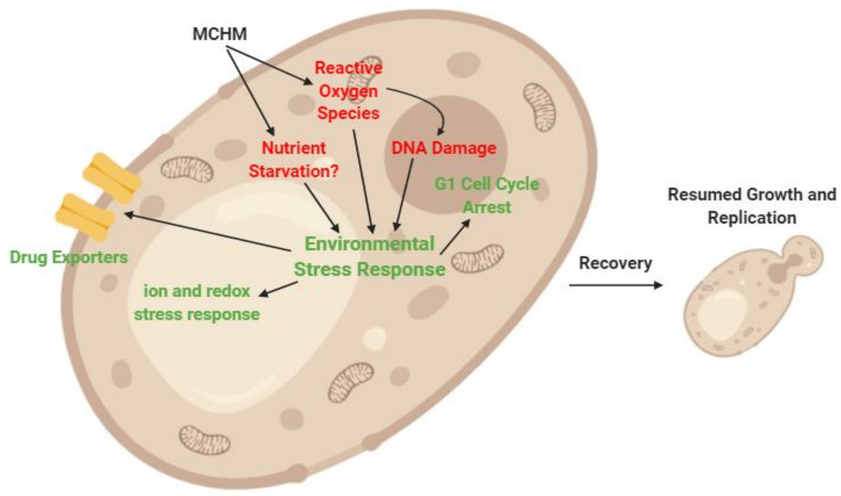


Figure 6 Comet Assay for DNA Damage. DNA damage produces double-strand breaks. Fragmented DNA can travel further under electrophoresis, so length of "comet" tails from cells under electric field corresponds to extent of DNA damage. Hydrogen peroxide was used as a positive control.

*550ppm MCHM > 5mM H202 (p = 0.0002)

**1000ppm MCHM > 5mM H202 (p = 0.0025)

Model of MCHM Tolerance



Acknowledgments

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