# Quantitative transcriptome, proteome, and sulfur metabolite profiling of the *Saccharomyces cerevisiae* response to arsenite

Thorsen, M., Lagniel, G., Kristiansson, E., Junot, C., Nerman, O., Labarre, J., & Tamás, M. J. (2007). Quantitative transcriptome, proteome, and sulfur metabolite profiling of the *Saccharomyces cerevisiae* response to arsenite. Physiological genomics, 30(1), 35-43. DOI: 10.1152/physiolgenomics.00236.2006

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**Sulfiknights**:

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# An overview of *S. cerevisiae* in response to arsenic

- Toxic substances, such as arsenic, have impacts on living organisms
- Met4p and Yap1p are major players in the cells' response to arsenic
- Experimental methods reveal the transcriptional regulation of genes
- The stimulation of sulfur assimilation and GSH biosynthesis is important for arsenic tolerance

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# Arsenic is toxic, but this toxicity can be used to treat acute cancers

- In Bangladesh and West Bengal, arsenic contaminates drinking water through the earth's geographic supply.
  - Chronic exposure to arsenic can cause different medical threats including cardiovascular diseases, neurological disorders, and liver injury.
- Despite its harmful consequences, arsenic trioxide is used to treat acute promyelocytic leukemia (blood and bone marrow).

# *Saccharomyces cerevisiae* experiences stress from arsenic metalloid

- *Saccharomyces cerevisiae* can resist arsenic through the regulation of cytosolic redox and glutathione levels.
- Certain transcription factors like Yap8p, control the expression of *ACR2* and *ACR3,* which are genes that possess arsenic-specific detoxification properties.
- The glutathione (GSH) biosynthesis pathway is essential for building resistance against oxidative stress and metal toxicity.

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# Met4p & Yap1p

- Met4p plays a role in cadmium tolerance. It controls sulfur assimilation and GSH biosynthesis. Met4p plays a role in AS(III) tolerance but its role in cadmium tolerance is more important
- Yap1p controls transcription of genes encoding proteins. It contributes to YCF1 and ACR3
  - Acr3 sequesters glutathione-conjugated As(III) in a vacuole and Ycf1p transports it as well as helps in reducing As(III) influx

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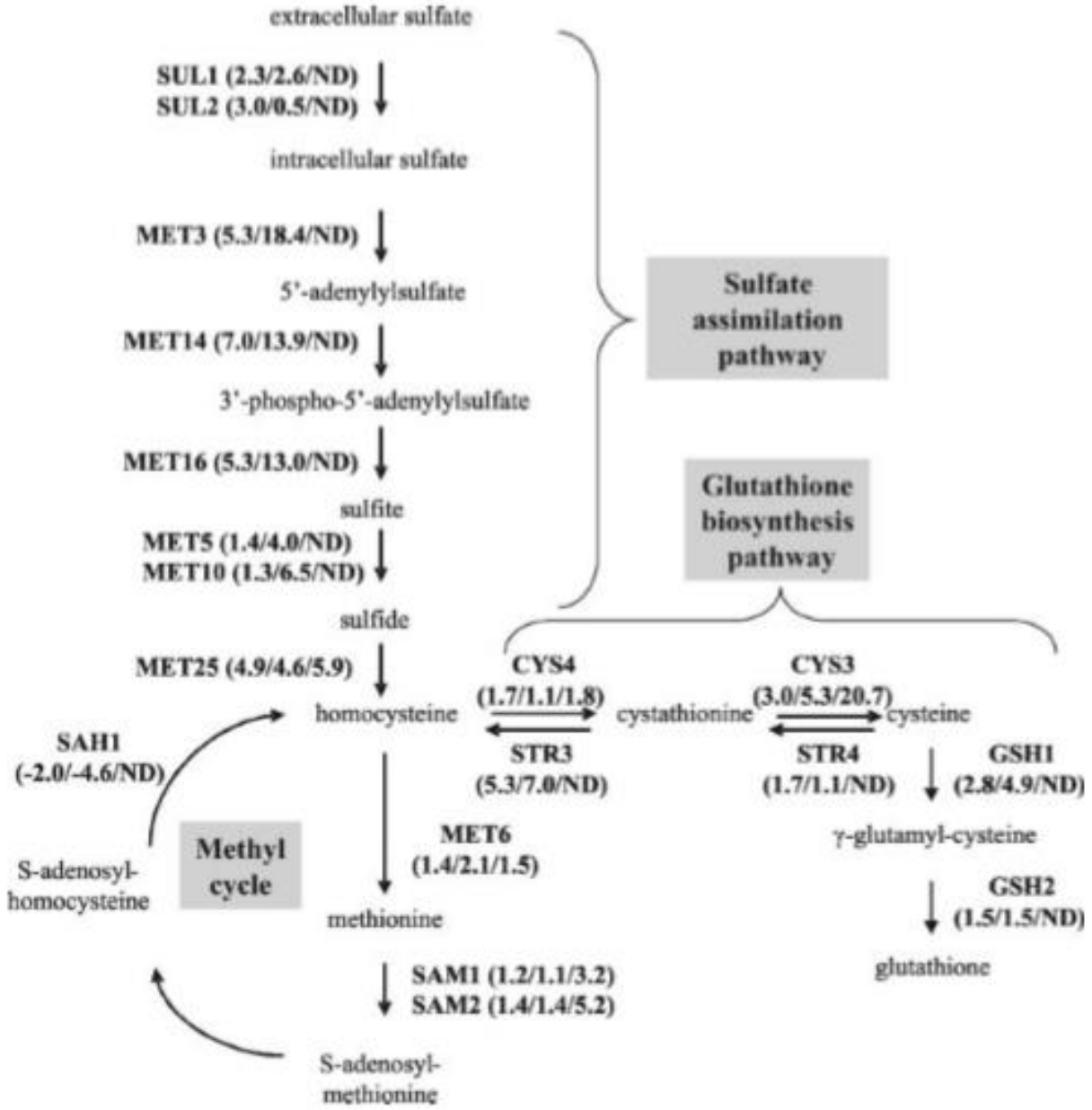


**Figure 1**: This figure gives us the flow chart extracellular sulfate must go through in order to become assimilated and ultimately assist in the Glutathione biosynthesis pathway. Sulfate must be converted to sulfide, which will enter the Methyl cycle and the glutathione biosynthesis pathway before it finishes as GSH2, or glutathione.

| Strain | Genotype | Source |
|---|---|---|
| W303-1A | *MAT***a** *ura3-1 leu2-3/112 trp1-1 his3-11/15 ade2-1 can1-100 GAL SUC2 mal0* | Ref. 32 |
| RW124 | W303-1A *yap1*Δ::*loxP* | Ref. 40 |
| CC849-1B | *MAT***a** *his3 leu2 trp1 ura3 met4*Δ::*TRP1* | Ref. 29 |
| RW104 | W303-1A *acr3*Δ::*loxP-kanMX-loxP* | Ref. 29 |
| YPDahl166 | W303-1A *acr3*Δ::*KanMX met4*Δ::*TRP1* | Present study |

**Table 1**: This table presents the different stains that were utilized through this experiment. W303-1A, RW124, CC849-1B and RW104 information was obtained from previous source material, but YPDagl166 was used in the present study at hand.

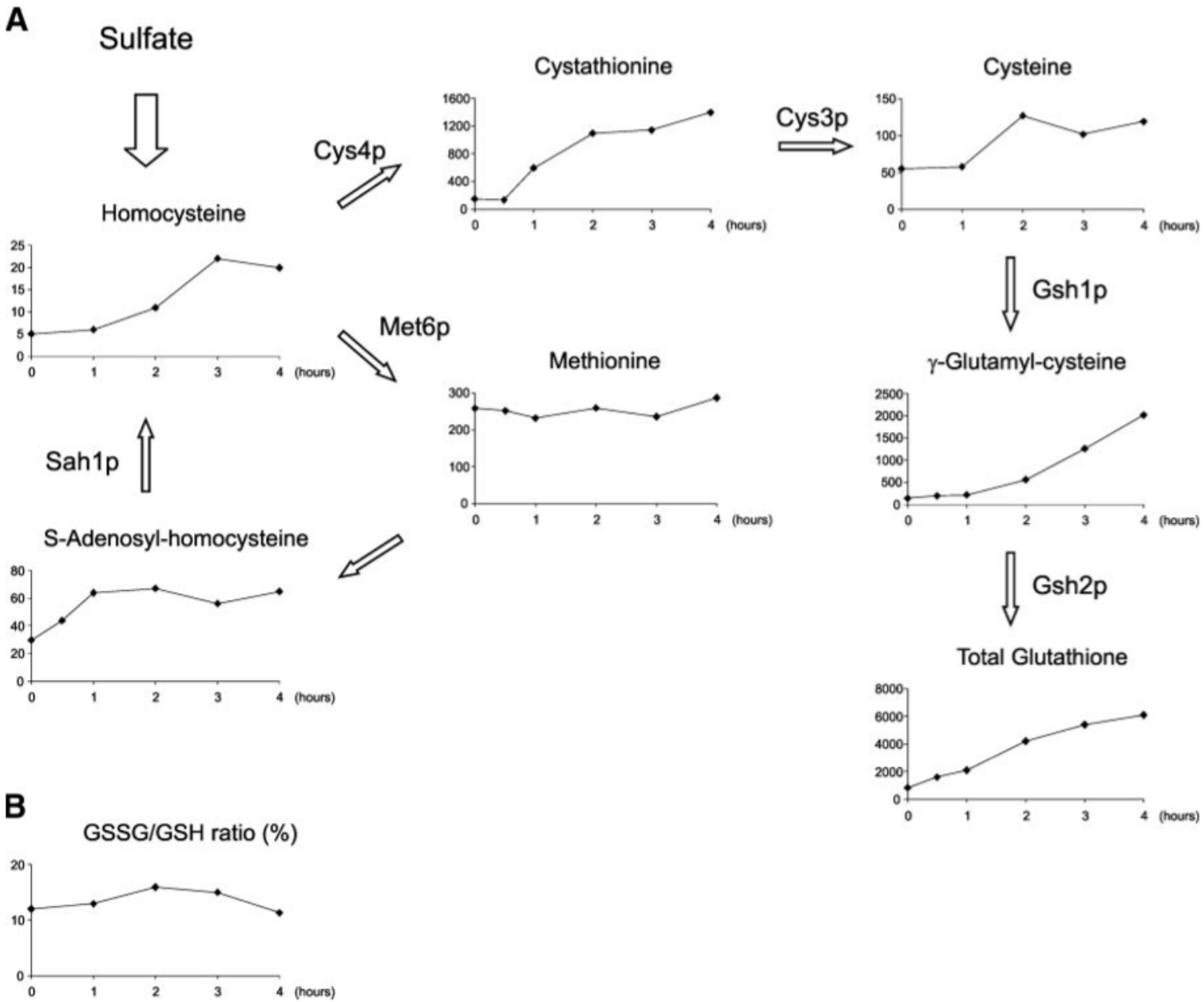


**Figure 2**: This figure shows the kinetic response of sulfur metabolites in response to arsenite (0.2mM). It also shows the ratio of oxidized to reduced GSH over time.

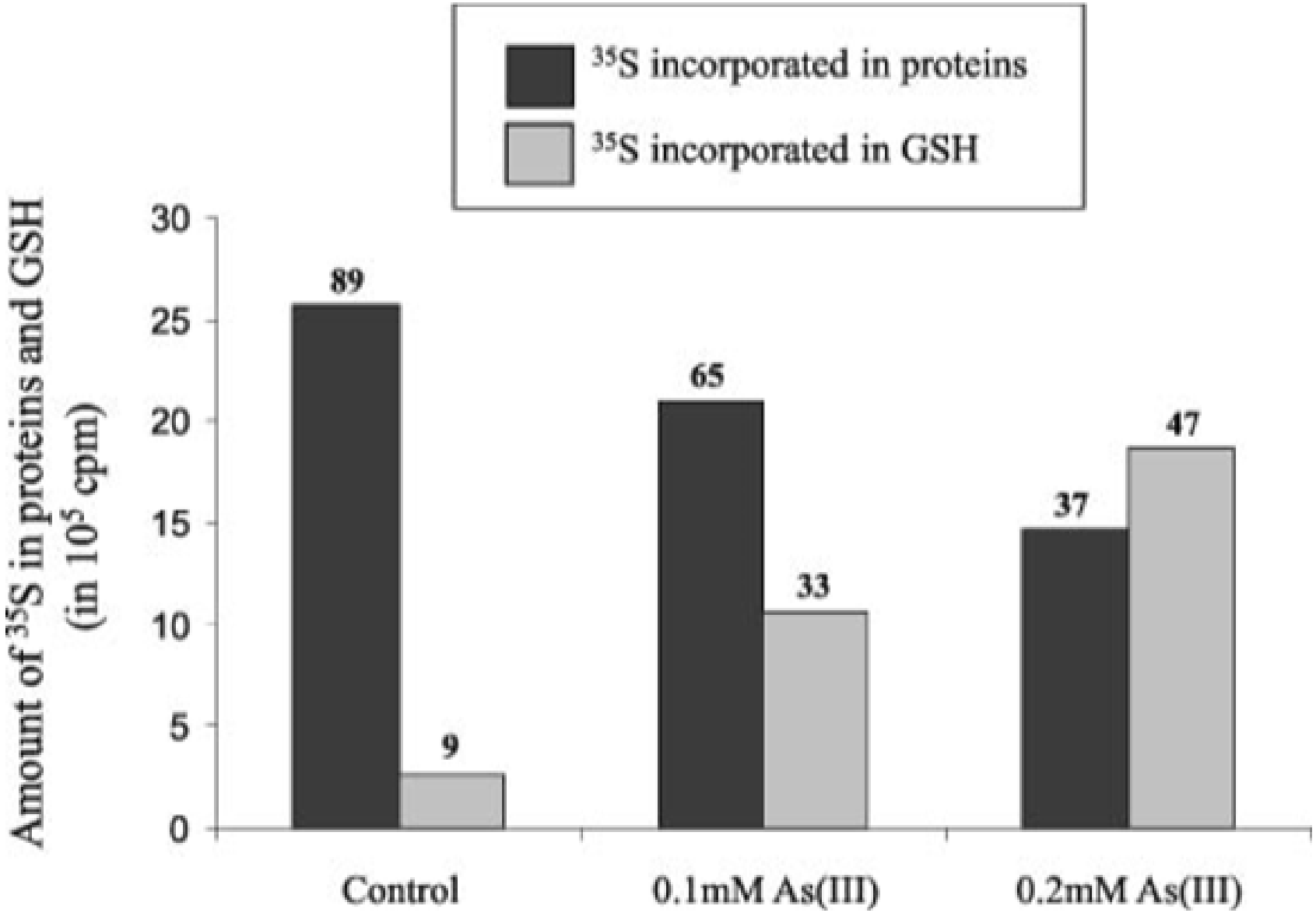


**Figure 3**: This shows the rise in GSH in response to increased concentration of As(III). Numbers at the top of the bars represent the percentage of assimilated sulfate in GSH proteins.

| Transcription Factor | *P* Value | Target Genes Encode Proteins with Function in . . . |
|---|---|---|
| *Transcription factors with overrepresented DNA binding site in the promoters of upregulated genes* | | |
| Rpn4p | $1.94 \times 10^{-23}$ | Proteasome function |
| Yap2p (Cad1p) | $9.12 \times 10^{-11}$ | Stress response |
| Yap7p | $2.91 \times 10^{-09}$ | Unknown |
| Msn4p | $6.36 \times 10^{-09}$ | Environmental stress response |
| Msn2p | $6.36 \times 10^{-09}$ | Environmental stress response |
| Yap3p | $6.65 \times 10^{-09}$ | Unknown |
| Yap5p | $6.65 \times 10^{-09}$ | Unknown |
| Yap4p (Cin5p) | $6.65 \times 10^{-09}$ | Unknown |
| Yap1p | $2.14 \times 10^{-06}$ | Resistance to oxidative stress |
| Adr1p | $2.17 \times 10^{-05}$ | Peroxisomal function, utilization of alternative carbon sources |
| Met32p | $1.34 \times 10^{-04}$ | Sulfur metabolism |
| Met31p | $1.34 \times 10^{-04}$ | Sulfur metabolism |
| Cbf1p | $3.64 \times 10^{-03}$ | Sulfur metabolism |
| *Transcription factors with overrepresented DNA binding site in the promoters of downregulated genes* | | |
| Sfp1p | $5.92 \times 10^{-21}$ | Ribosomal function |
| Rap1p | $4.61 \times 10^{-17}$ | Ribosomal function |
| Fhl1p | $3.33 \times 10^{-08}$ | Ribosomal function |
| Spt23p | $1.31 \times 10^{-05}$ | Unknown |
| Aft2p | $1.33 \times 10^{-05}$ | Iron homeostasis, resistance to oxidative stress |
| Hap1p | $6.27 \times 10^{-04}$ | Response to cellular heme and oxygen levels |

**Table 2**: This table displays the genes with transcripts found to be upregulated by more than twofold, 13 transcription factors were found to have highly overrepresented binding motifs, while of the genes with transcripts found to be downregulated by at least twofold, 6 transcription factors were found to have highly overrepresented binding motifs

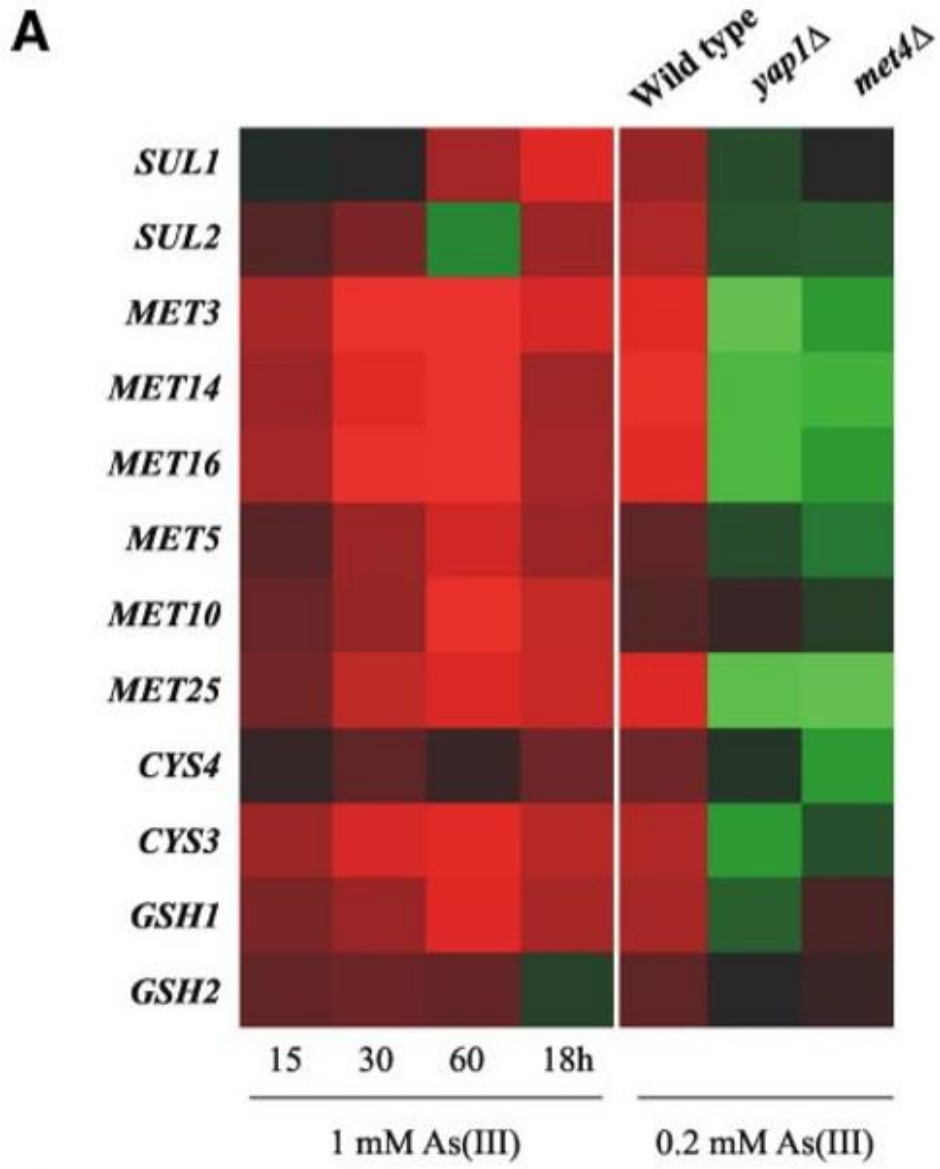


Figure 4: Heat map showing expression changes after 15min, 30min, 60min, and 18hrs of exposure to 1mM As(III), and the role of Yap1 and Met4 expression after 1hr of exposure to 0.2mM As(III)

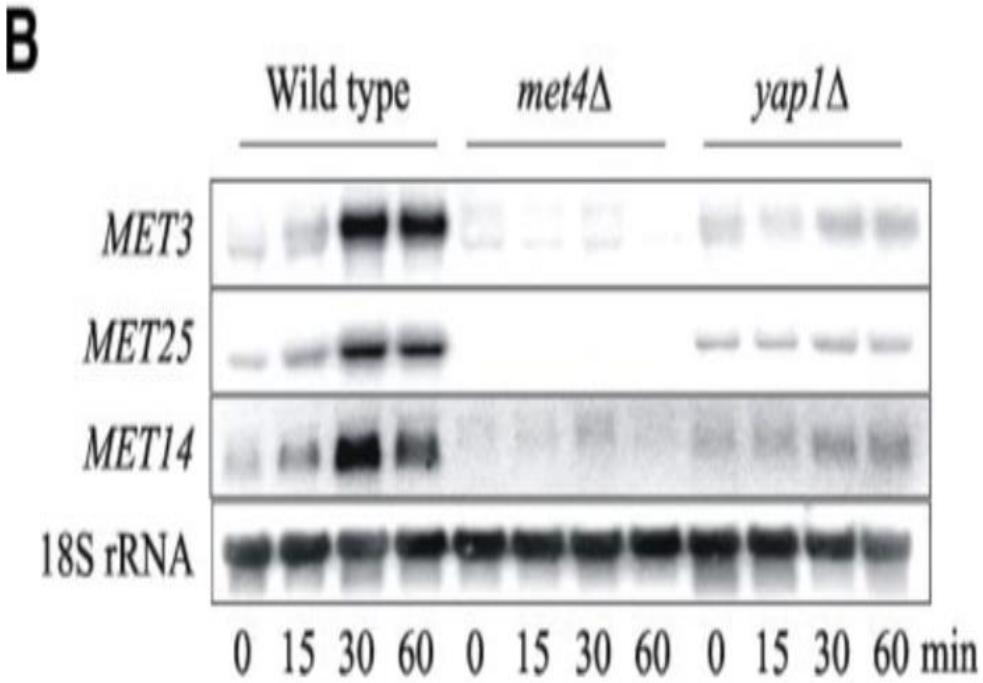


**Figure 4B**: This figure shows the expression of genes in RNA extracted from wildtype, met4Δ, and yap1Δ cells at certain time points after being exposed to 0.2 mM As(III). The X axis showed the time points at which the genes were analyzed for expression and the Y axis shows three MET genes being analyzed with 18s rRNA being the loading control. The measurements were made using 32P-labeled genes and a common trend of the Figure was that wild-type cells show high expression of their genes after exposure to As(III) than mutant met4Δ and yap1Δ cells.

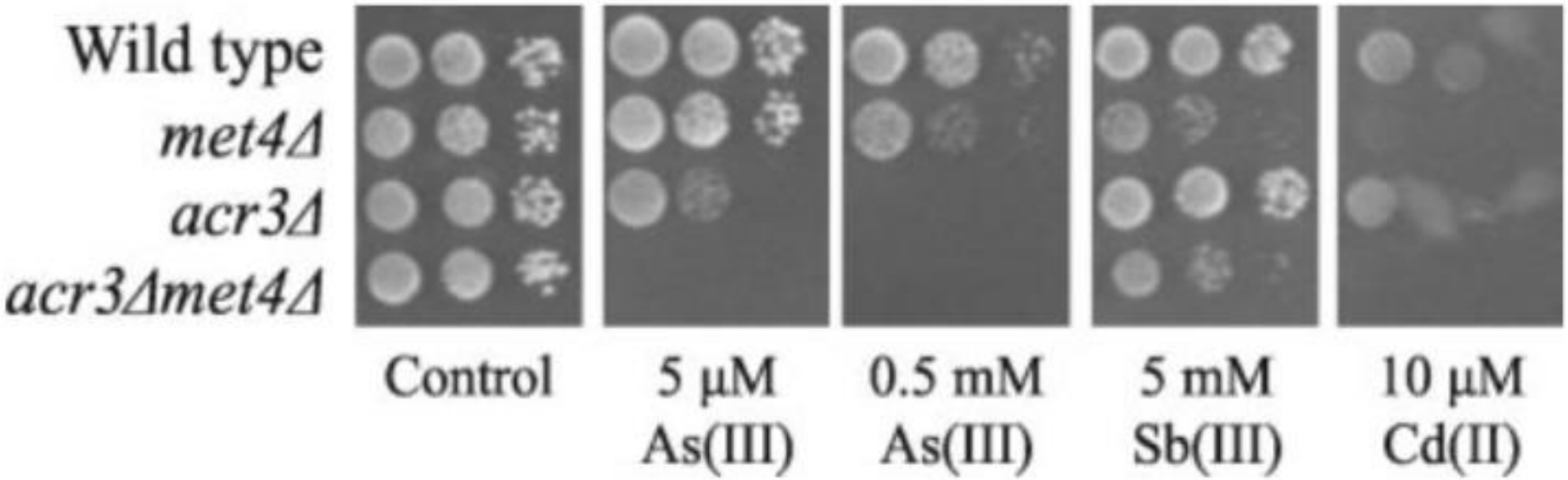


**Figure 5**: This figure shows the serial dilutions of wildtype, met4 mutant, acr3 mutant, and acr3met4 mutant. There was no observed growth on acr3met4 mutant plates in the presence of Sb(III) or Cd(II). Growth was monitored after 203 days at 30 °C

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# Stimulation of the sulfur assimilation/GSH biosynthesis pathways is important for cellular arsenic tolerance acquisition

- Controlled by *YAP1*p and Met4p transcription factors
- Higher concentration of arsenic caused a greater concentration of sulfur metabolites
- Sulfur is incorporated in glutathione biosynthesis in higher amounts in the presence of arsenite. During arsenic presence there is less sulfite found in proteins
- Mutations in *met4* and *arc3* had a negative impact with the formation of cell cultures
- Acr3 is necessary to defend against AS(III) when the cell does not have the necessary processes to undergo trivalent arsenite efflux

# Source used for this presentation


- Thorsen, M., Lagniel, G., Kristiansson, E., Junot, C., Nerman, O., Labarre, J., & Tamás, M. J. (2007). Quantitative transcriptome, proteome, and sulfur metabolite profiling of the Saccharomyces cerevisiae response to arsenite. Physiological genomics, 30(1), 35-43. DOI: 10.1152/physiolgenomics.00236.2006
- (2006). Cammack, R., Atwood, T., Campbell, P., Parish, H., Smith, A., Vella, F., & Stirling, J. (Eds.), Oxford Dictionary of Biochemistry and Molecular Biology. : Oxford University Press. Retrieved 13 Nov. 2019, from https://www.oxfordreference.com/view/10.1093/acref/9780198529170.001.0001/acref-9780198529170.

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