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chaired by Prof. Peter Meikle, Dr. Thusitha W. Rupasinghe, Prof. Susan Sumner, Dr. Katja Dettmer-Wilde
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The Use of Mitochondrial Metabolomics via Combined GC/LC-MS Profiling to Reveal Metabolic Dysfunctions in *sym1*-deleted Yeast Cells

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Introduction

SYM1…

- is an ortholog of human *MPV17*, whose mutation causes mitochondrial DNA depletion syndrome [1]
- encodes a channel protein, which is located in the inner mitochondrial membrane [2,3]

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However, the function of sym1/mpv17 protein is still **unknown**. Their deletion or mutation results in…

- impaired mitochondrial bioenergetics functions and morphological features [4]
- insufficiency of deoxynucloetide and slow DNA replication in mitochondria [5]

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Hypothesis about sym1/mpv17…

- Sym1 channel transports metabolic intermediates into and out of mitochondria [4]
- Mpv17 is a weakly cation-selective channel that modulates membrane potential[6]

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Introduction

Mitochondrial metabolomics

- Isolation of mitochondria and the rest of cytoplasm before metabolomics analysis
- Compartment-specific distribution and regulation of metabolites could be observed [7, 8]

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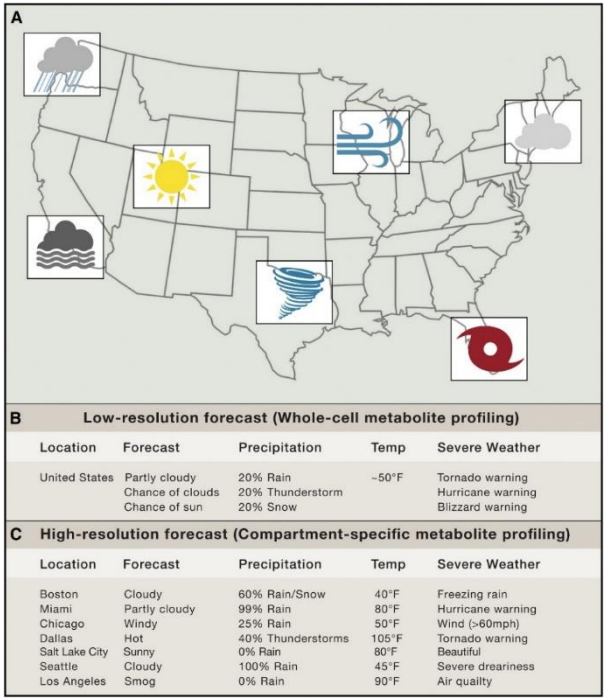
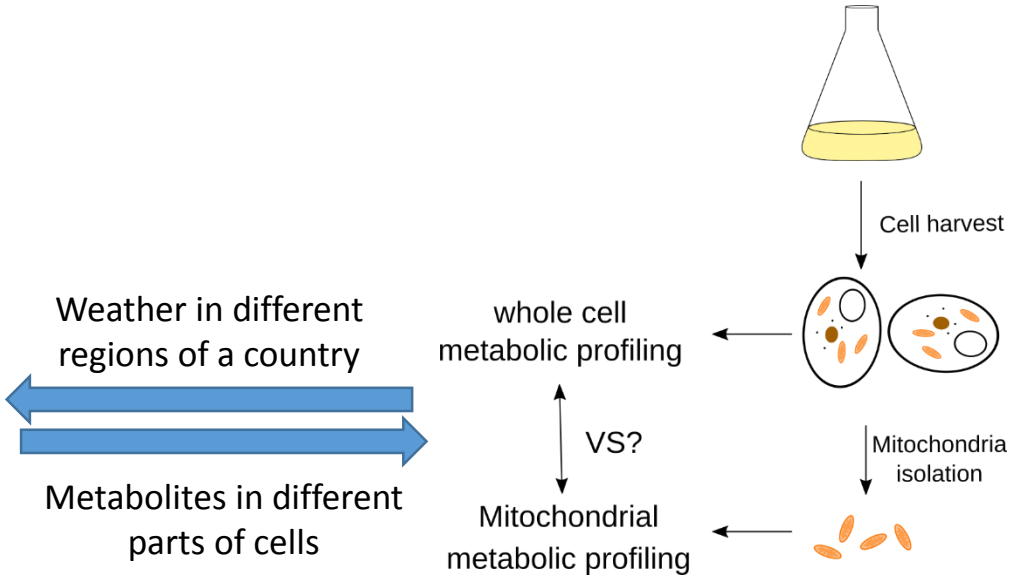


B Low-resolution forecast (Whole-cell metabolite profiling)

Location	Forecast	Precipitation	Temp	Severe Weather
United States	Partly cloudy	20% Rain	~50°F	Tornado warning
	Chance of clouds	20% Thunderstorm		Hurricane warning
	Chance of sun	20% Snow		Blizzard warning

C High-resolution forecast (Compartment-specific metabolite profiling)

Location	Forecast	Precipitation	Temp	Severe Weather
Boston	Cloudy	60% Rain/Snow	40°F	Freezing rain
Miami	Partly cloudy	99% Rain	80°F	Hurricane warning
Chicago	Windy	25% Rain	50°F	Wind (>60mph)
Dallas	Hot	40% Thunderstorms	105°F	Tornado warning
Salt Lake City	Sunny	0% Rain	80°F	Beautiful
Seattle	Cloudy	100% Rain	45°F	Severe dreariness
Los Angeles	Smog	0% Rain	90°F	Air quailty



[9] Van Vranken and Rutter, 2016

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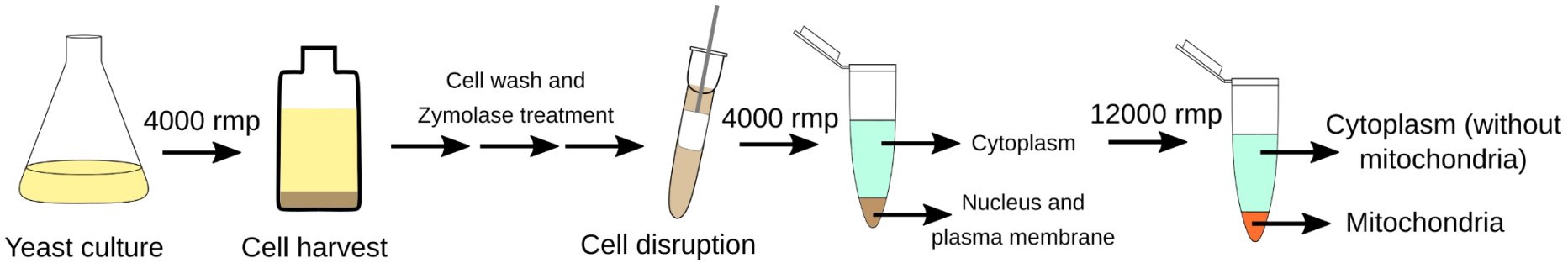
Workflow

Mitochondria isolation



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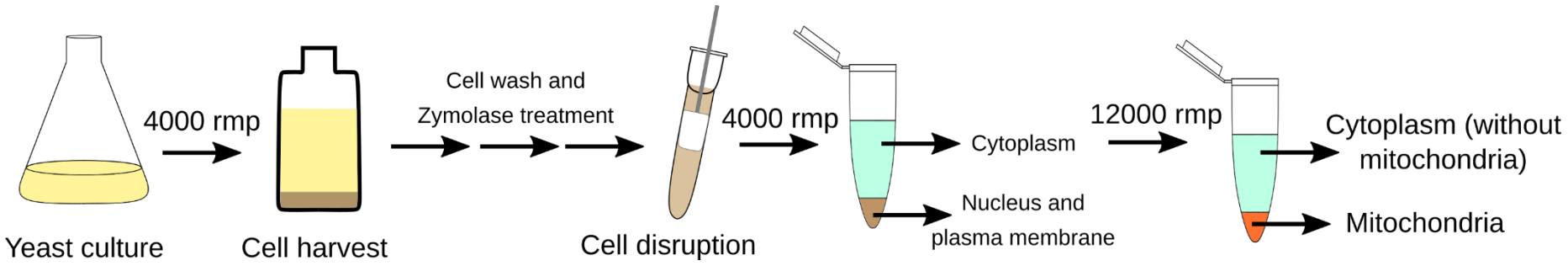
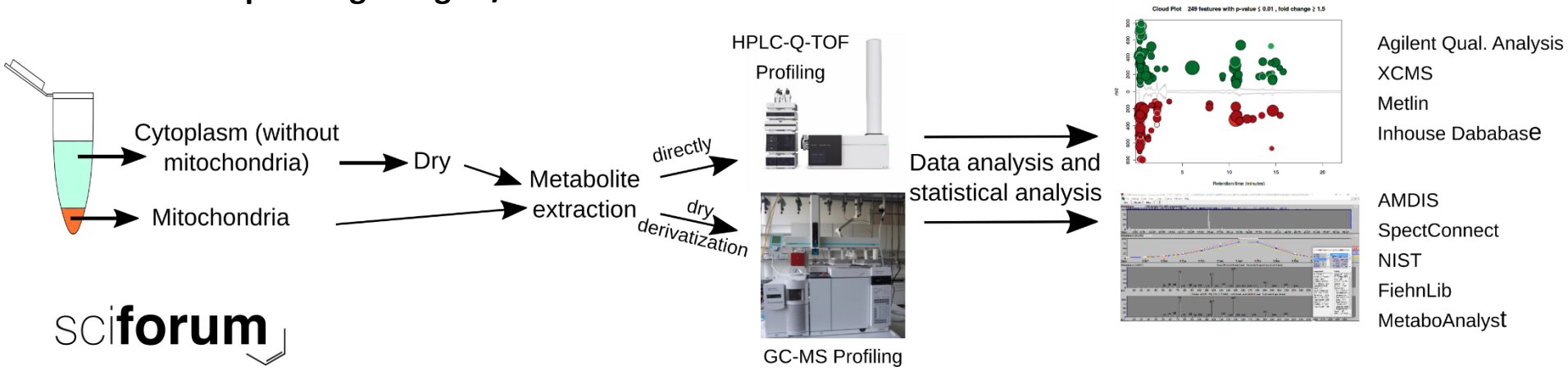
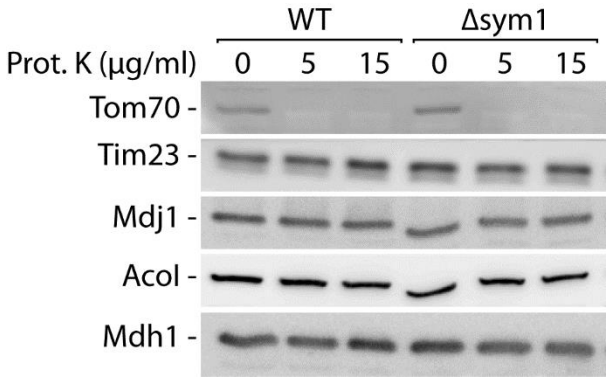


Metabolic profiling using LC/GC-MS



Results and Discussion

Mitochondrial inner membrane was intact after the isolation



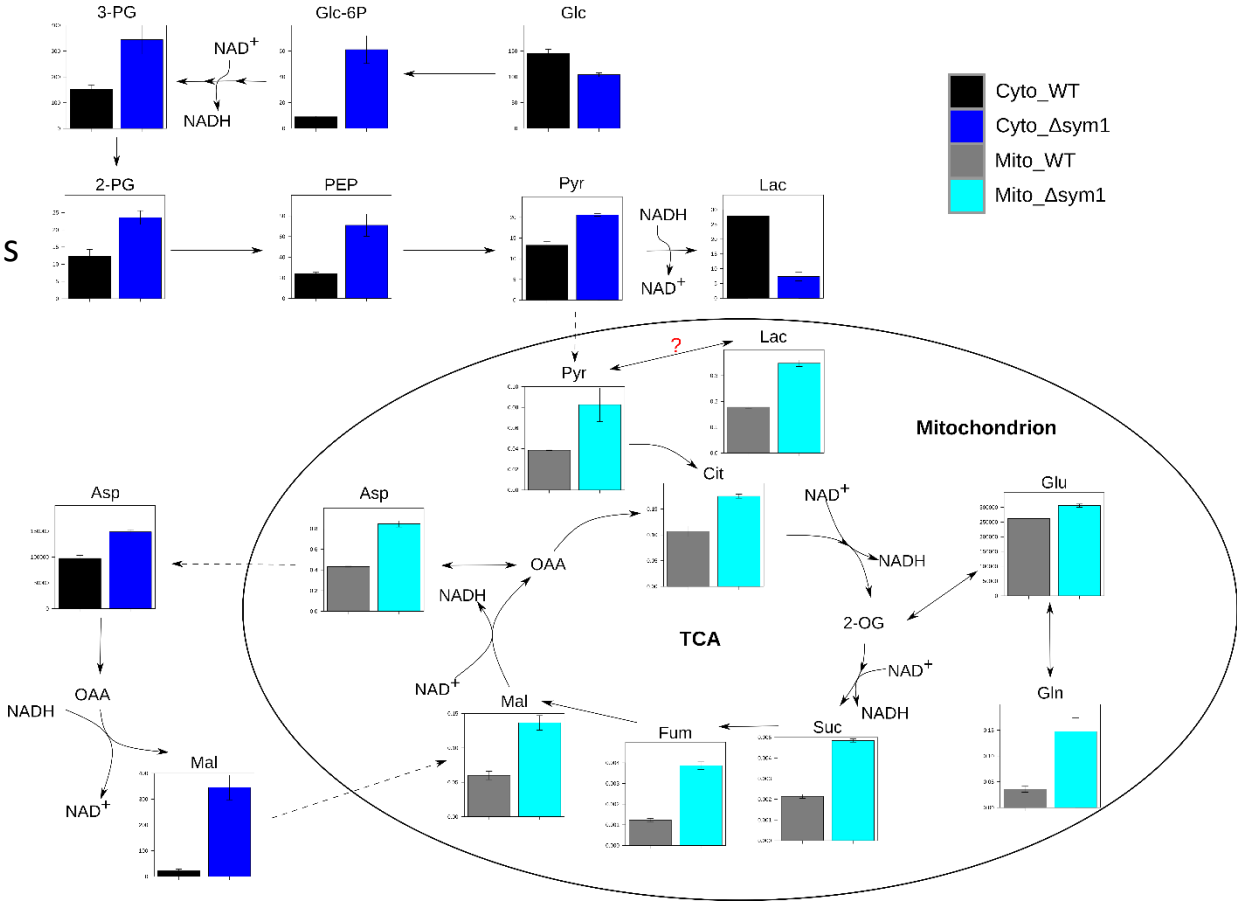
After proteinase K incubation Tom70 were depleted, while Tim23 and other marker proteins were maintained, indicating an intact mitochondrial inner membrane.

Tom: protein translocase of mitochondrial outer membrane
Tim: protein translocase of mitochondrial inner membrane
Mdj: mitochondrial DnaJ (HSP40) family
Aco: aconitase
Mdh1: mitochondrial malate dehydrogenase

Results and Discussion

Impaired energy metabolism in Δ*sym1* cells

- Upregulated/accumulated glycolysis and TCA cycle intermediates



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Impaired energy metabolism in Δ*sym1* cells

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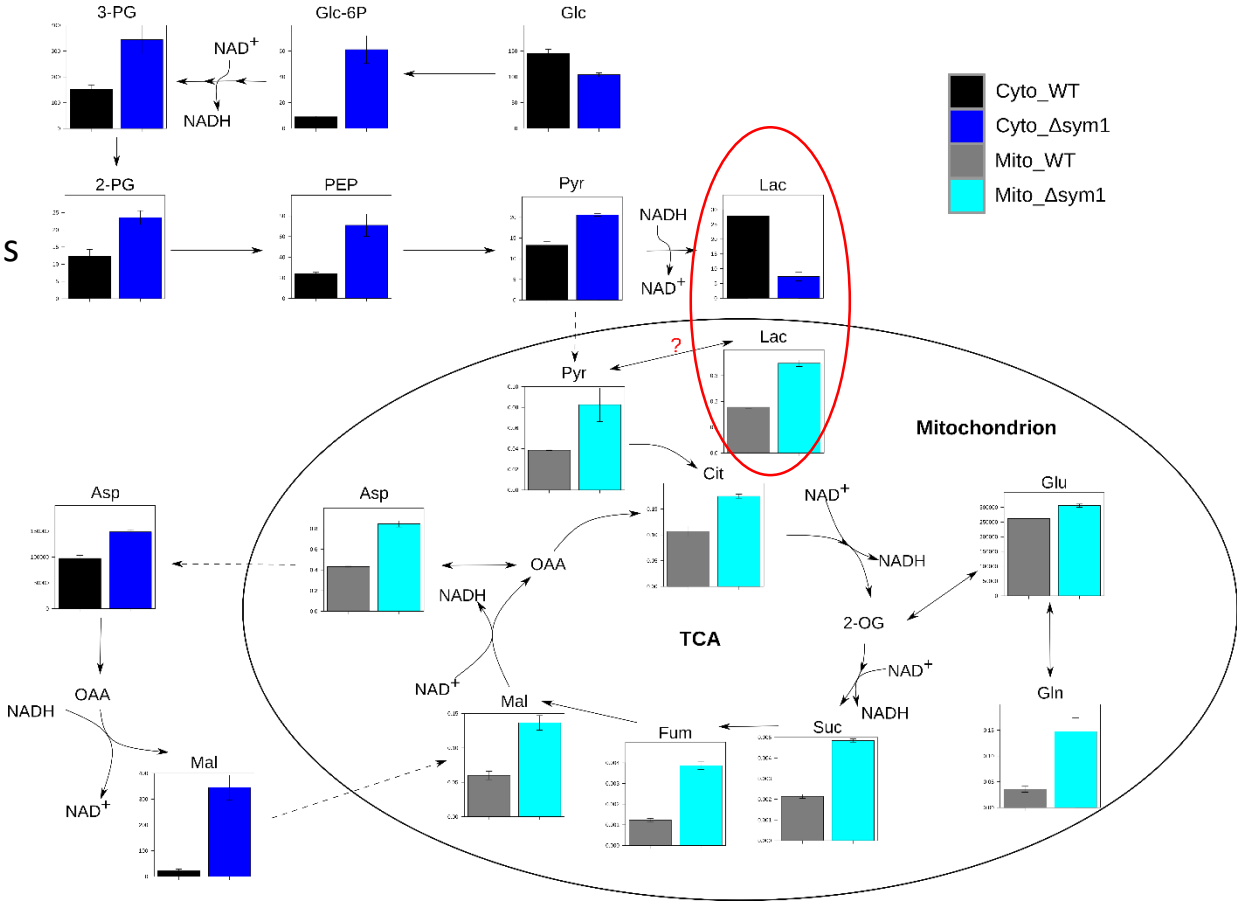
- Lactate was reduced in cytosol but accumulated in mitochondria



Results and Discussion

Impaired energy metabolism in Δ*sym1* cells

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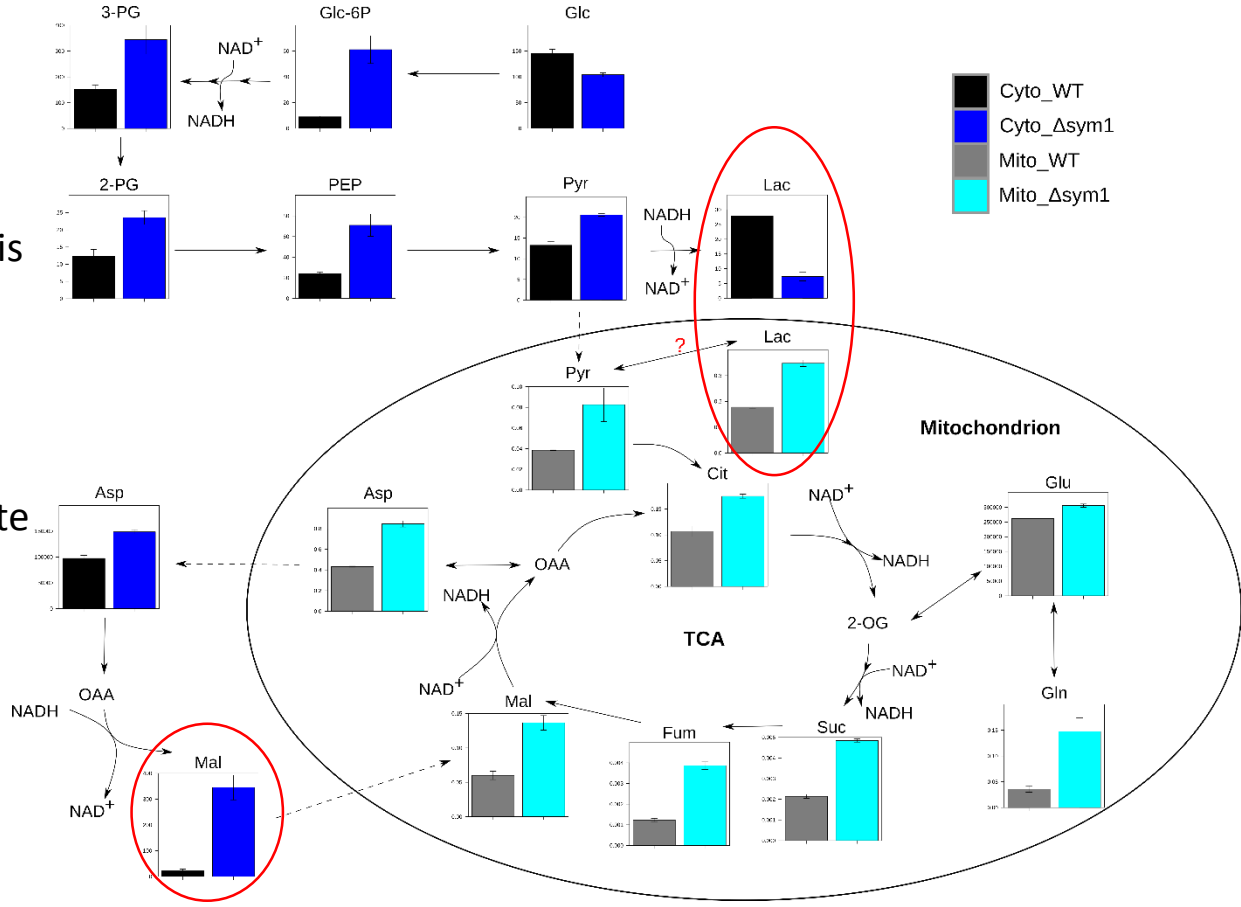
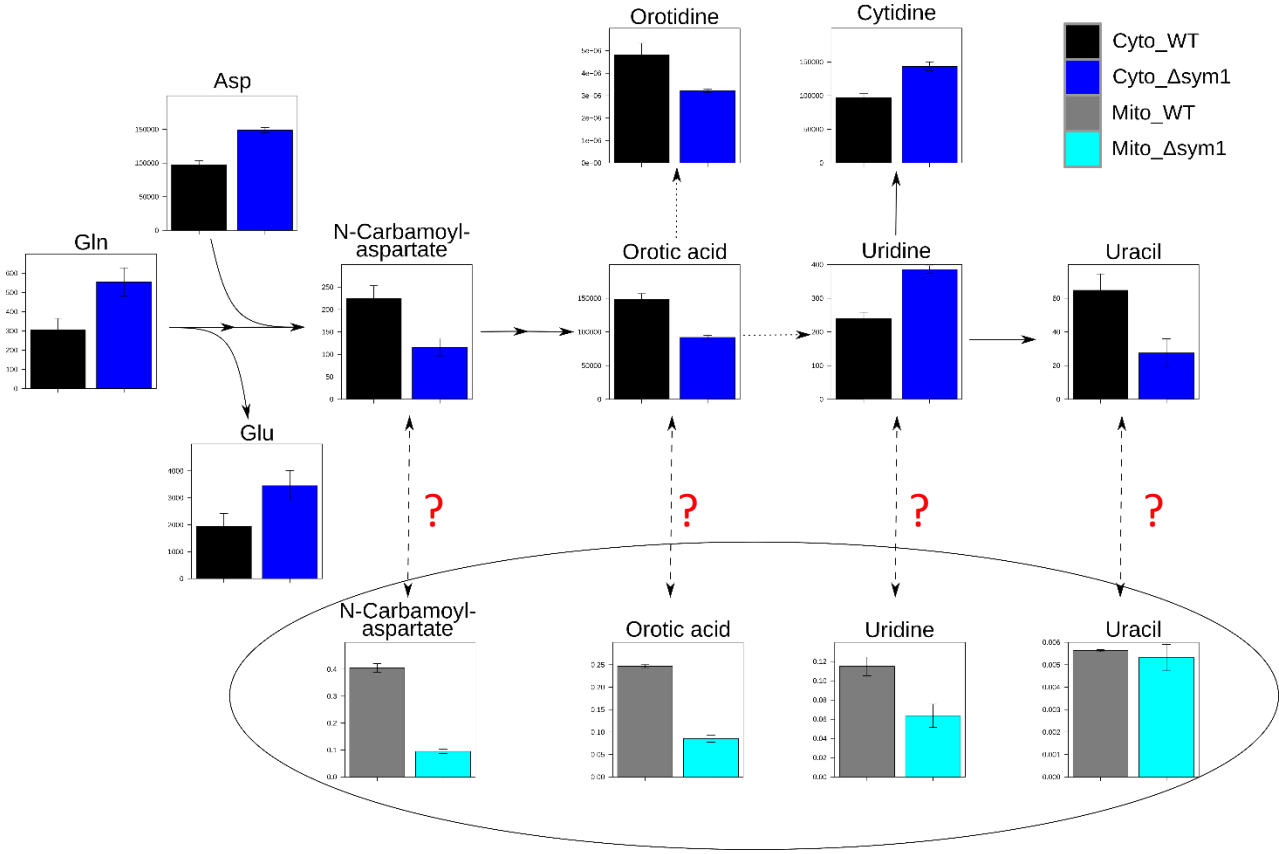
- Lactate was reduced in cytosol but accumulated in mitochondria
- Highly accumulated cytosolic malate



Results and Discussion

Impaired pyrimidine metabolism

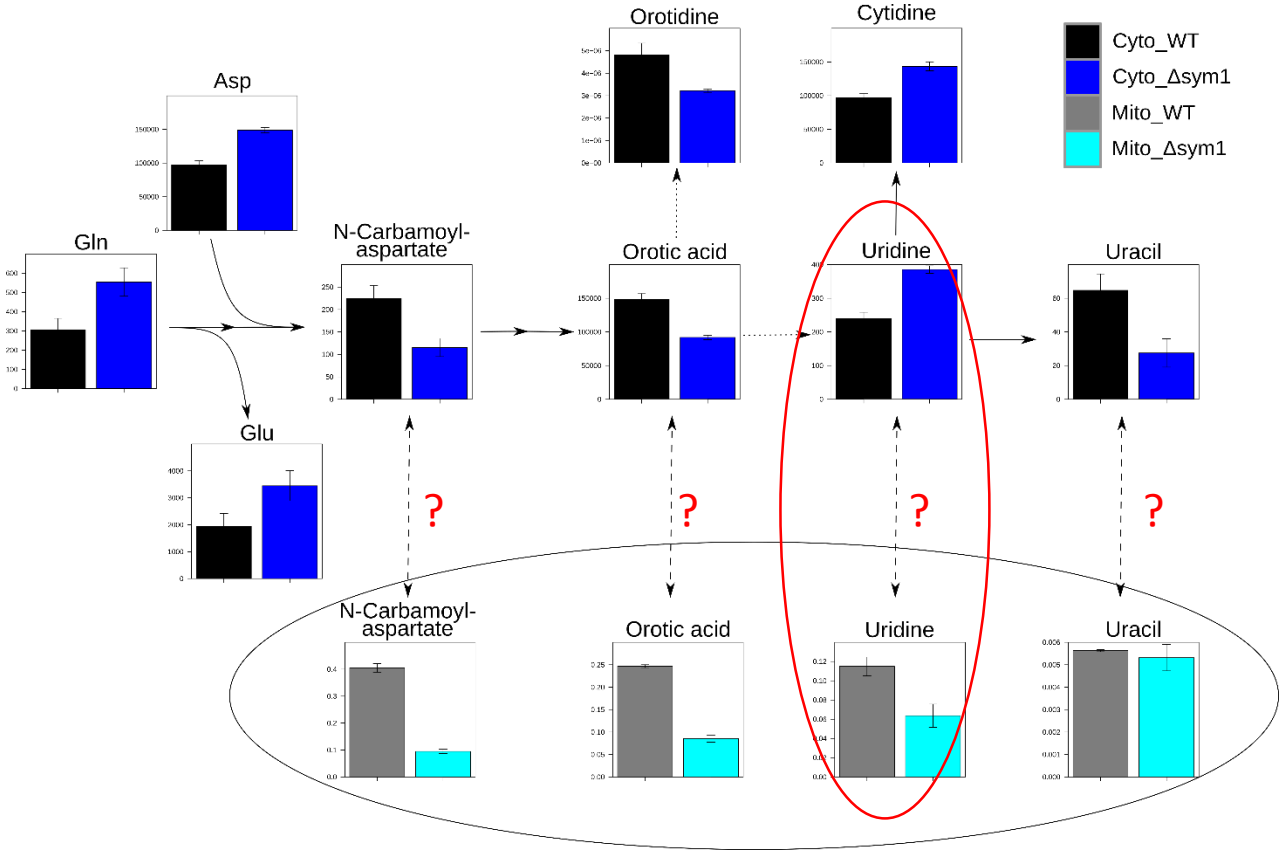
- Reduction of most of the pyrimidine biosynthesis intermediates



Results and Discussion

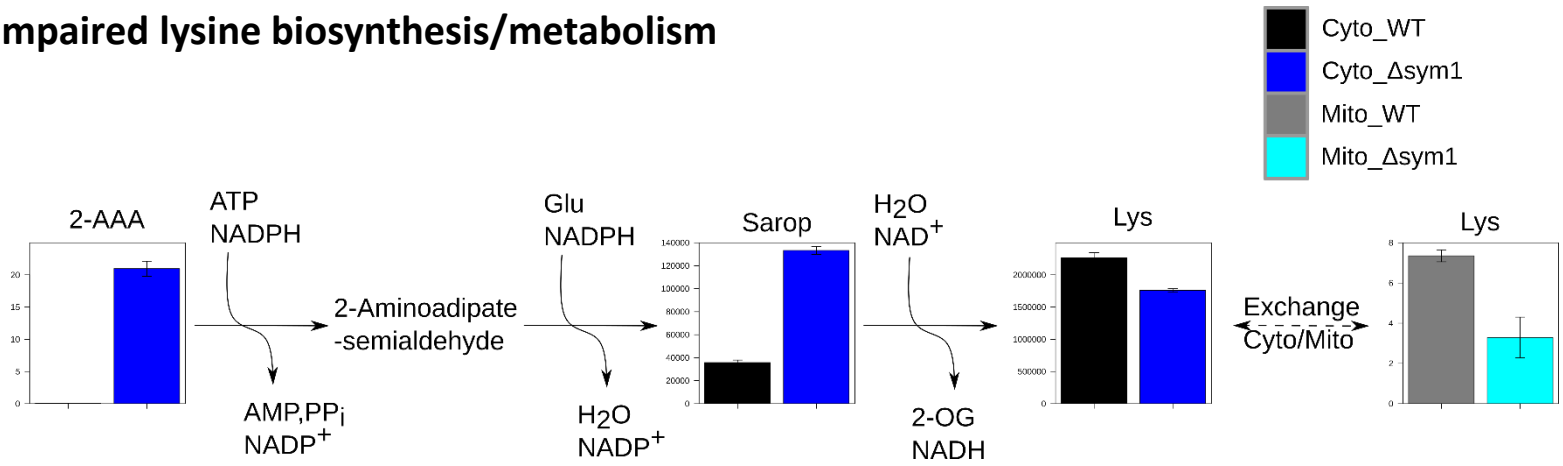
Impaired pyrimidine metabolism

- Reduction of most of the pyrimidine biosynthesis intermediates
- Accumulation of cytosolic uridine
- May be related to the mDNA deletion syndrome



Results and Discussion

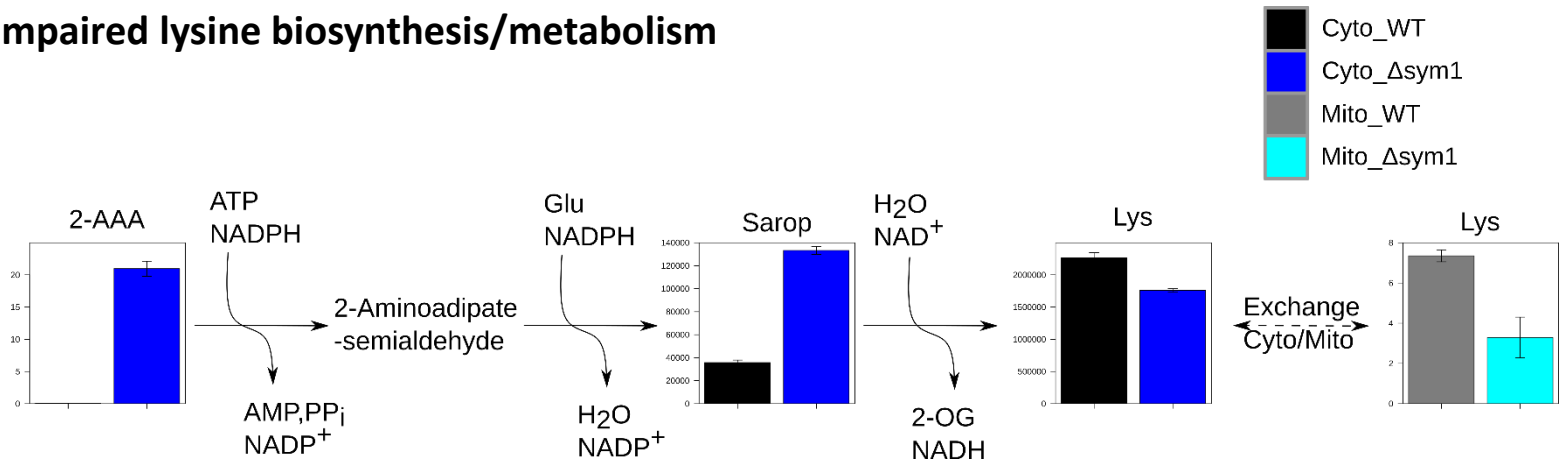
Impaired lysine biosynthesis/metabolism



Accumulation of 2-amminoadipic acid (2-AAA) and saccharopine (Sarop) and reduction of lysine indicate an interrupted lysine biosynthesis/metabolism.

Results and Discussion

Impaired lysine biosynthesis/metabolism



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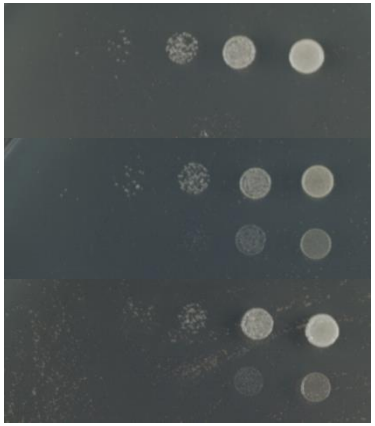
Given that cells can take up lysine from the medium, a drop test will be investigated to prove the hypothesis.

Results and Discussion

Δ*sym1* yeast cells have a defective lysine biosynthesis



SCGal (mimimal medium with galactose as carbon source) plates were incubated at 30 °C, 40 hours
Other plates at 19 °C and 23 °C showed the same effect. (data not shown here)

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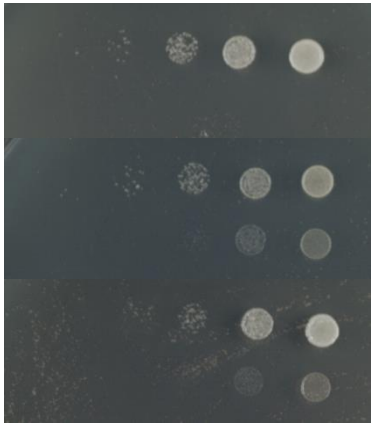

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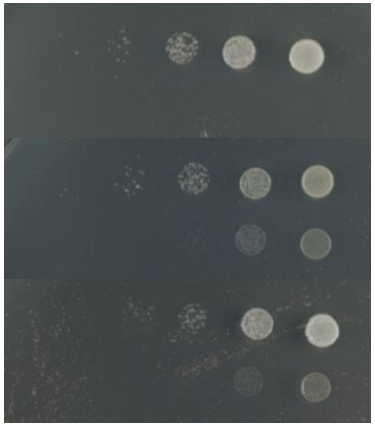

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Interestingly, sym1 protein is not correlated with lysine biosynthesis, so what can be the reason for this fatal effect?

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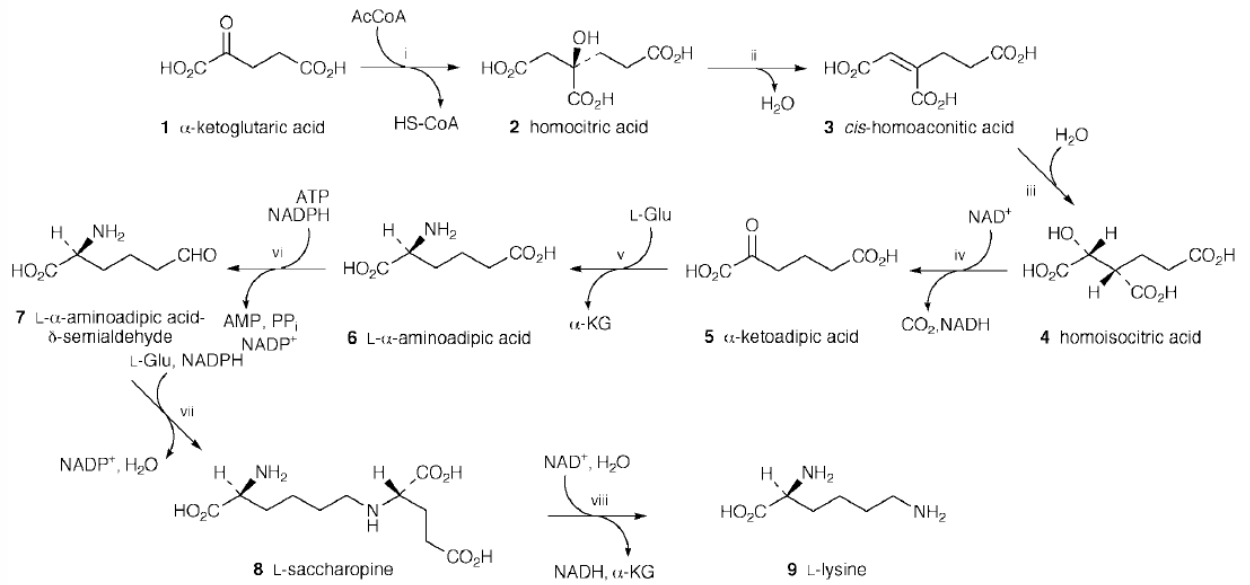
Results and Discussion

Yeast lysine biosynthesis



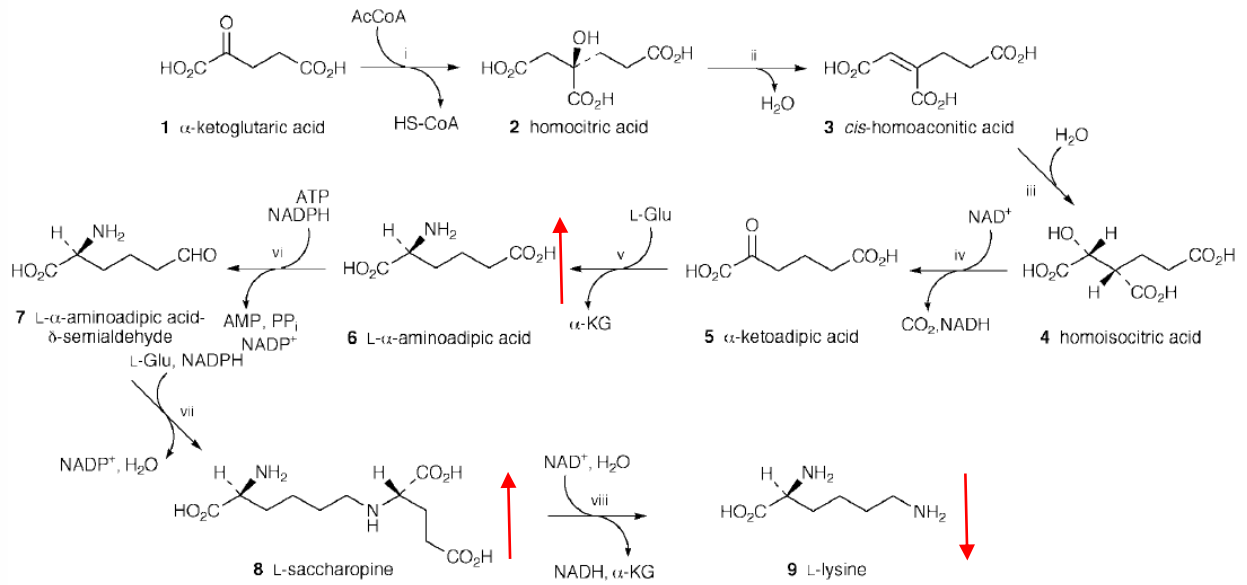
Scheme 1 Enzymes of the fungal α-aminoadipate pathway to lysine: i, homocitrate synthase EC 4.1.3.21; ii & iii homoaconitase EC 4.2.1.36; iv, homoisocitrate dehydrogenase EC 1.1.1.87; v, aminoadipate aminotransferase EC 2.6.1.39; vi, aminoadipate reductase EC 1.2.1.31; vii saccharopine reductase EC 1.5.1.10; viii, saccharopine dehydrogenase EC 1.5.1.7.

[10] Zabriskie and Jackson, 2000.

Results and Discussion

Potential reasons for the defective lysine biosynthesis in Δ*sym1* cells

- Accumulated intermediates and reduced lysine indicates a defect in the last step



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sciforum

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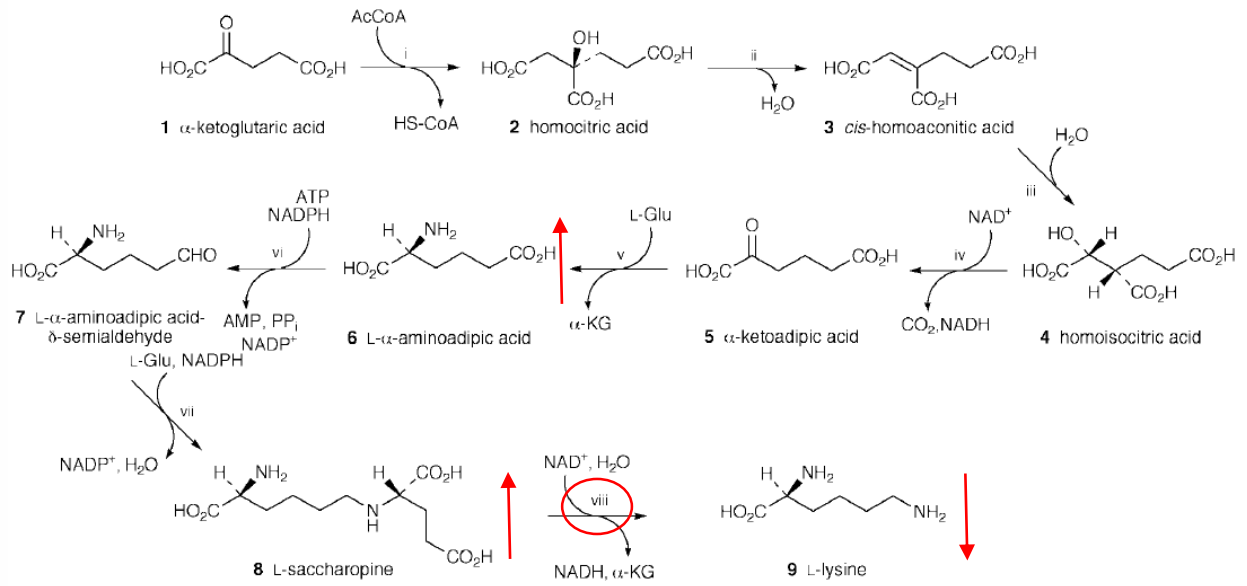
Results and Discussion

Potential reasons for the defective lysine biosynthesis in Δ*sym1* cells

- Inhibited or down-regulated saccharopine dehydrogenase



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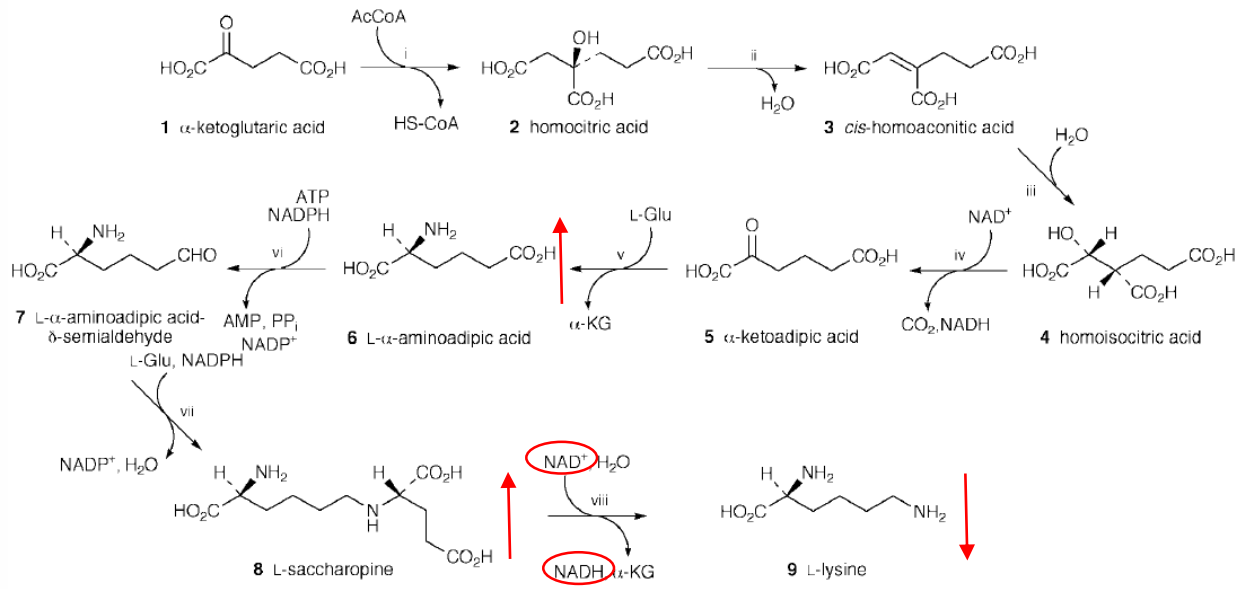
Results and Discussion

Potential reasons for the defective lysine biosynthesis in Δ*sym1* cells

- Changed redox state in cytosol results in reduced NAD^+ and accumulated NADH
- This is correlated with the accumulated glycolysis and reduced lactate
- NAD^+ is regenerated by reducing pyruvate to lactate, which is apparently inhibited in Δ*sym1* cells



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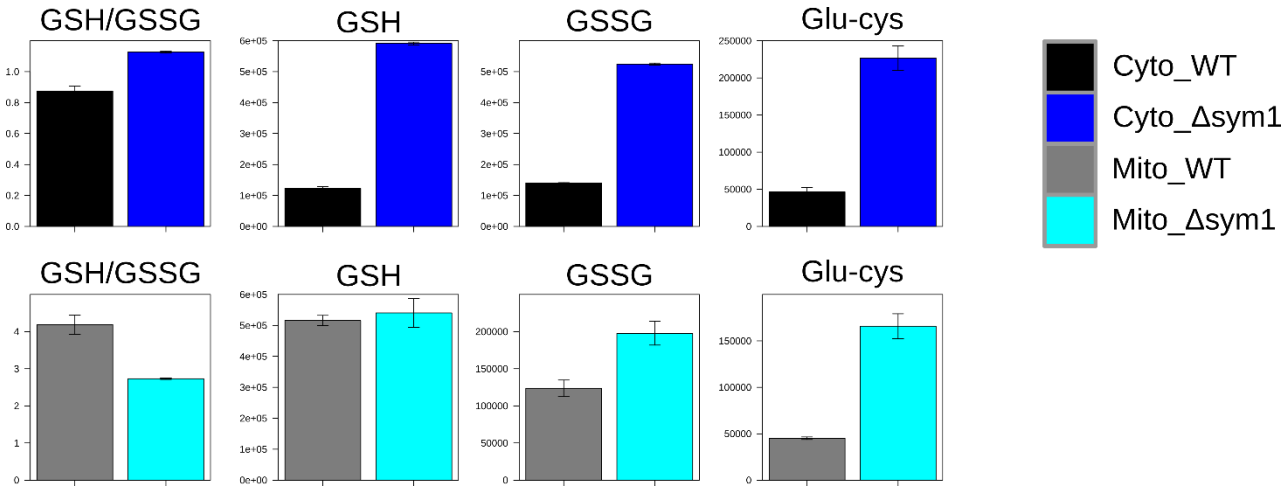
Altered glutahione metabolism indicates a imbalanced redox state



- Increased cytosolic GSH/GSSG ratio
- Reduced mitochondrial GSH/GSSG ratio
- Overall upregulated/accumulated glutathione fluxes

Outlook

- NAD(P)H assay to identify the Redox state in the mitochondria and cytosol
- Application of proteomics to interesting candidates
- Application of isotope-labeled metabolites to track the altered pathways
- Screening of about 50 unknown mitochondrial membrane protein knockouts
- Automatization the search of candidates by python script to find out significant altered metabolites from the screening

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Johannes Plagge

Lukas Braun

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