Minimal Glycolysis and Pathway Swapping in Saccharomyces cerevisiae

A Minimal Set of Glycolytic Genes Reveals Strong Redundancies in Saccharomyces cerevisiae Central Metabolism

and

Pathway swapping: Toward modular engineering of essential cellular processes

Kuijpers N.G.A., Solis-Escalante D., et al. (2015, 2016)

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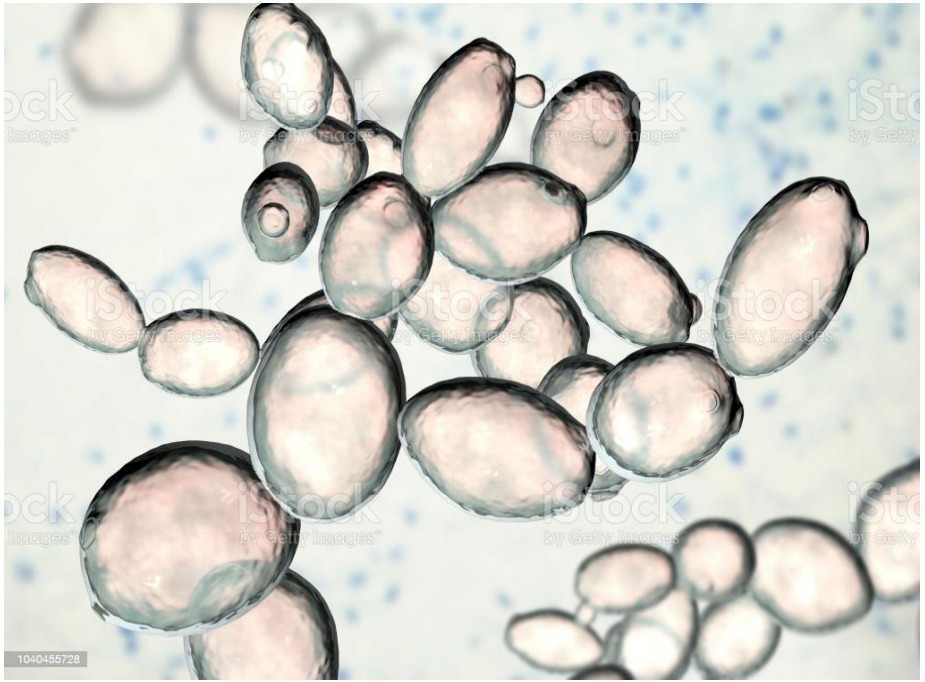
Content

- Background
- Introductions
  - Minimal glycolysis
  - Pathway swapping
- Minimal glycolysis
  - Aim of the study
  - Results
- Pathway swapping
  - Aim of the study
  - Results

Background

As a result of ancestral whole-genome and small-scale duplication events, the genomes of *Saccharomyces cerevisiae* and many eukaryotes still contain a substantial fraction of duplicated genes.

- *S. cerevisiae*'s are favoring fermentative metabolism even in the presence of oxygen and characterized by a high glycolytic capacity.
- 12 glycolytic reactions leading to the biochemical conversion from glucose to ethanol are encoded by 27 paralogs
- Replacement of petrochemistry by bio-based processes requires microbes with new capabilities.
- Using the glycolytic pathway enabling the “transplantation” of essential metabolic routes in the model and industrial yeast.



Introduction - Minimal glycolysis

- Gene duplication plays a key role in evolution by providing DNA templates for innovation, while preventing interference with the cellular function of the original genes. After gene duplication, the resulting paralog pairs are usually identical and therefore functionally redundant, duplicated genes will eventually be pseudogenized and lost from the genome.
- A whole-genome duplication event in an ancestor of *S. cerevisiae*, ca. 100 million years ago, was followed by loss of ca. 90% of the resulting gene duplications. Many surviving paralog pairs still exhibit a substantial degree of functional redundancy.
- The Embden-Meyerhof-Parnas pathway, the main route for oxidation of glucose to pyruvate in all eukaryotes and other organisms, is among the most slowly evolving metabolic pathways. Paralog families are found for the structural genes that encode the EMP enzymes. Under conditions of oxygen limitation or sugar excess, *S. cerevisiae* couples the EMP pathway to the fermentative production of ethanol via pyruvate decarboxylase and NAD+-dependent alcohol dehydrogenase.
- In *S. cerevisiae*, no fewer than 8 of the 12 enzyme reactions in glycolysis are represented by multiple paralogous genes.

Introduction - Pathway swapping

- Radical remodelling of central metabolism could greatly accelerate fundamental and applied research, but is impeded by the mosaic organization of microbial genomes.

- Construction of a “single-locus glycolysis” *Saccharomyces cerevisiae* platform enabled quick and easy replacement of 26 glycolytic isoenzymes by any alternative, functional glycolytic pathway configuration.

- Growth depended on two nonnative glycolytic pathways:

  complete glycolysis from the related yeast *Saccharomyces kudriavzevii*

  mosaic glycolysis consisting of yeast and human enzymes

- Potential of modular, combinatorial approaches to engineering and analysis of core cellular processes.

Aim of the study

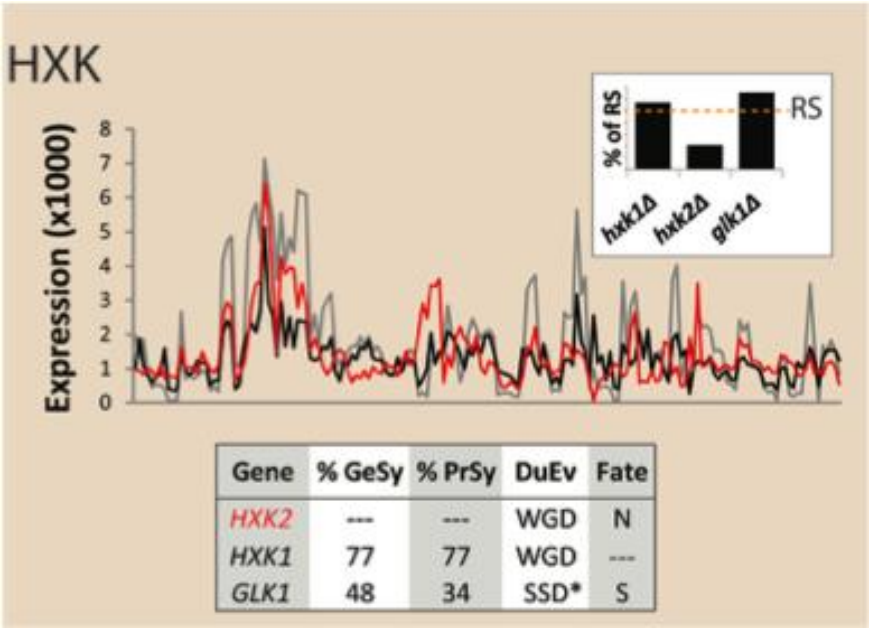
- Experimentally exploring the genetic redundancy in yeast glycolysis
- Done by deleting the “excessive”, minor paralogs from the glycolytic pathway
- Impact studied with two steps:
  - Glycolytic flux under a number of controlled conditions
  - Phenotype of the MG strain under wide array of experimental conditions
- Goals:
  - Aiding in the design of synthetic genomes
  - Formulation of mathematical models

Strain construction

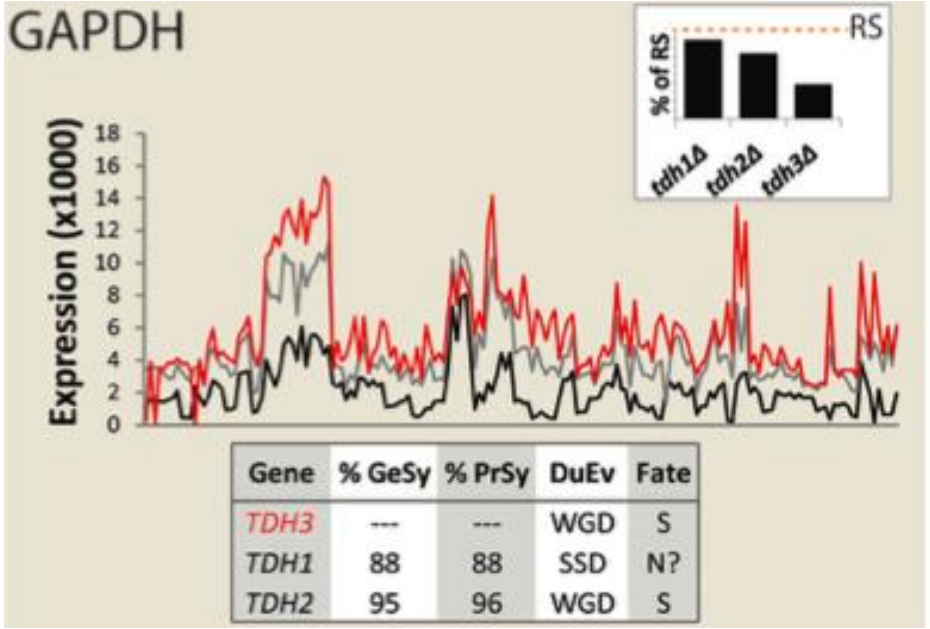
- Identification of major paralogs, 4 criteria:
  - Highest transcript level over a range of growth conditions
  - Most extensive loss of enzyme activity in cell extracts upon deletion
  - Moonlighting functions with a strong impact on specific growth rate or robustness
  - Strongest decrease in specific growth rate upon deletion
- 11 Major paralogs : *HXK2*, *PGI*, *FBA1*, *TPI1*, *TDH3*, *GPM1*, *ENO2*, *PGI1*, *PYK1* (*CDC19*), *PDC1*, and *ADH1*
- *PKF1* and *PFK2* work as subunits, deletion of either one decreases fitness substantially
- *ADH3* was retained to maintain growth rates under anaerobic circumstances

→ 13 minor paralogs deleted, 14 major paralogs retained

Major and minor paralogs



Gene	% GeSy	% PrSy	DuEv	Fate
HXK2	---	---	WGD	N
HXK1	77	77	WGD	---
GLK1	48	34	SSD*	S

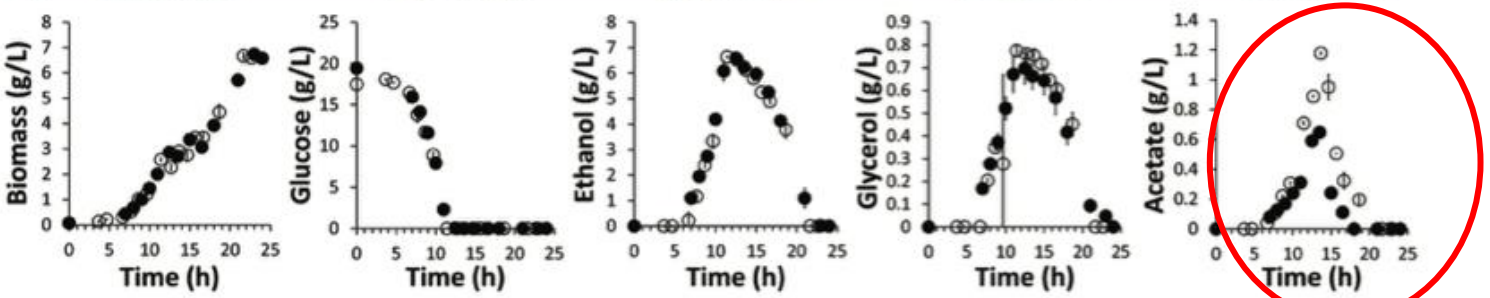


Gene	% GeSy	% PrSy	DuEv	Fate
TDH3	---	---	WGD	S
TDH1	88	88	SSD	N?
TDH2	95	96	WGD	S

Results - controlled conditions

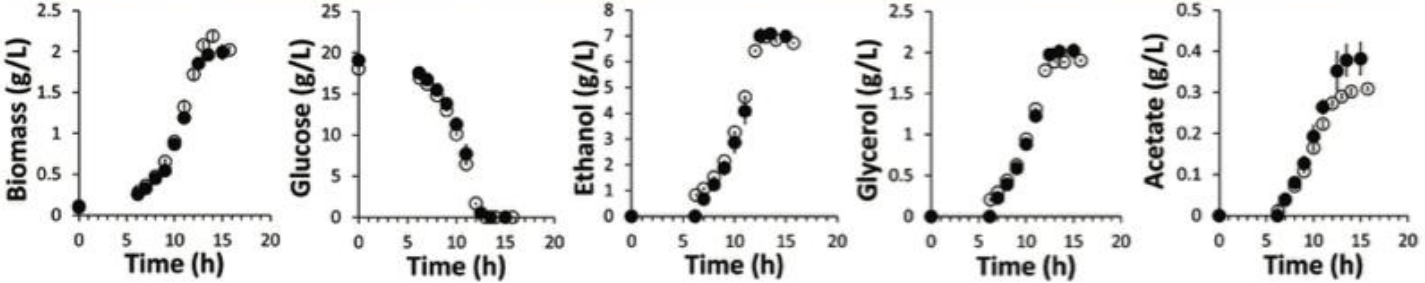
A

	μ^*	q_s	q_{EtOH}	q_{Glyc}	q_{Ace}^*
MG	0.357±0.013	15.362±0.578	21.748±2.097	1.177±0.077	0.632±0.144
C	0.335±0.011	15.770±0.451	23.816±1.068	1.113±0.031	0.411±0.066

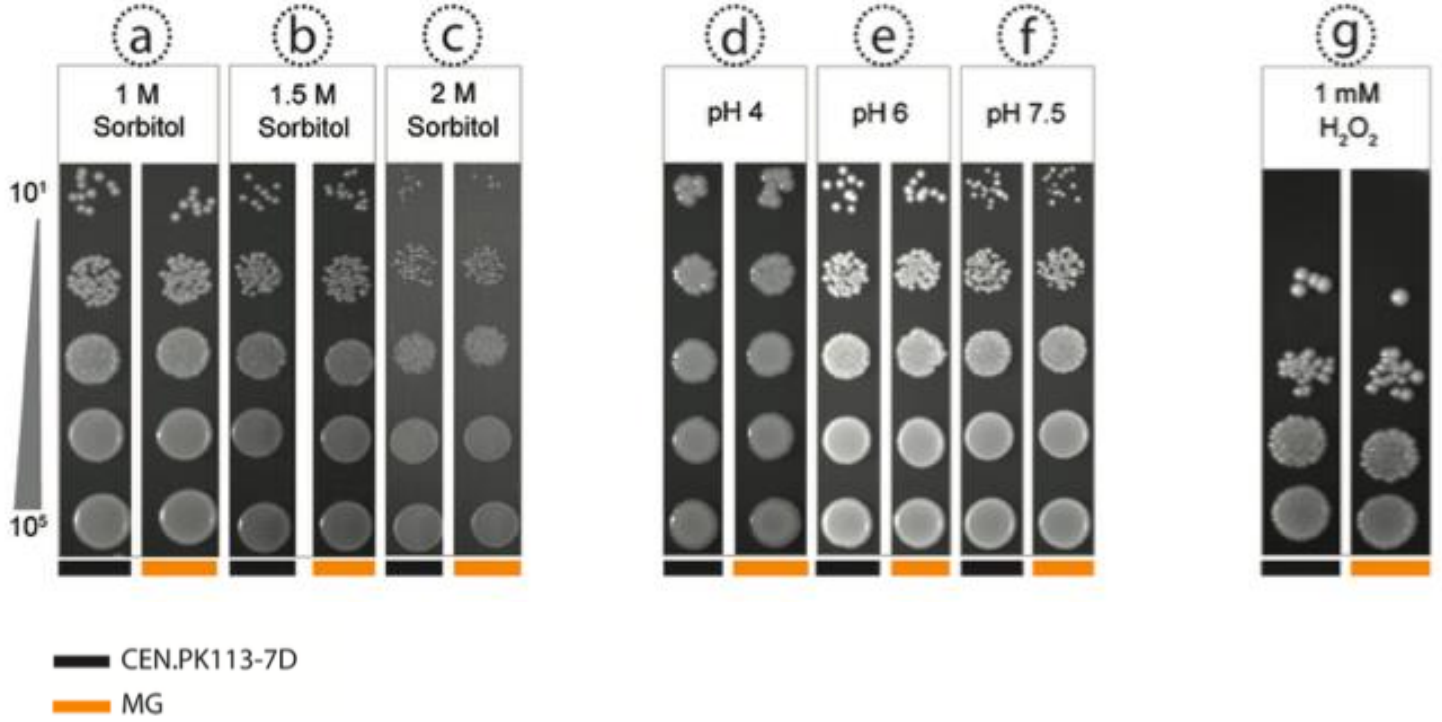


B

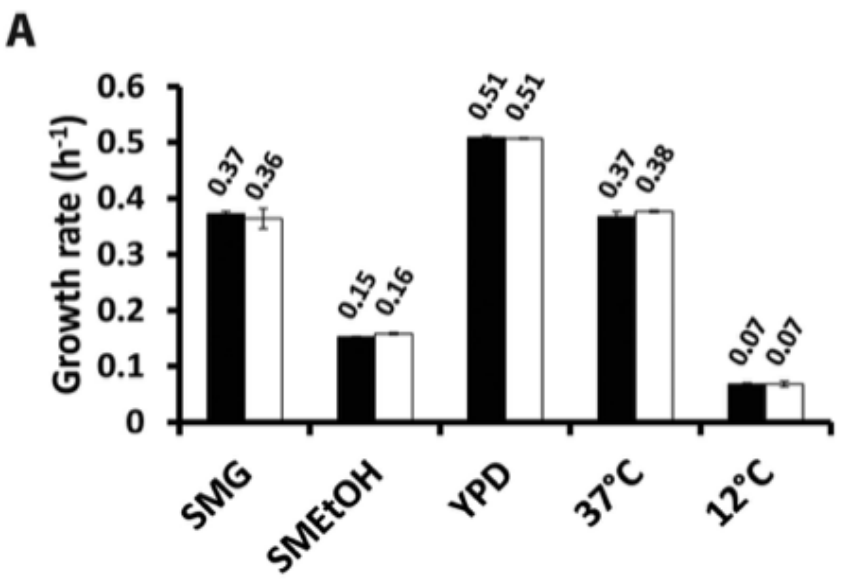
	μ	q_s	q_{EtOH}	q_{Glyc}	q_{Ace}
MG	0.332±0.009	17.039±2.179	27.130±1.814	3.731±0.199	0.420±0.047
C	0.319±0.017	18.427±0.301	27.904±0.121	3.870±0.268	0.403±0.123

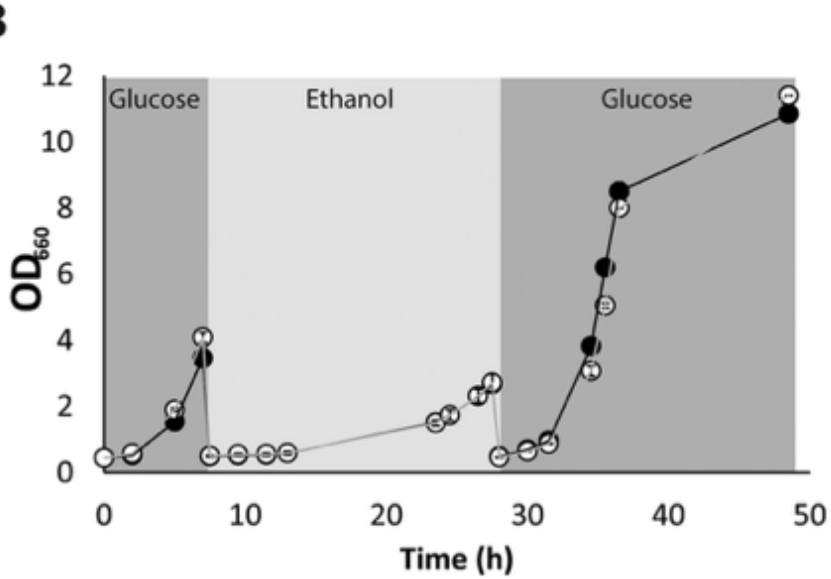


Results - experimental conditions, solid media



Results - experimental conditions, shake flasks





Conclusions

- Minimal changes in growth kinetics, intracellular metabolite concentrations, and gene expression compared to the congenic strain
    - only exception acetate production, which was slightly higher in the MG strain
- No specific phenotype was found for the MG strain under a wide range of conditions
    - Tested conditions represent only a fraction of possible conditions in the nature
- No specific phenotype under laboratory conditions makes it a great platform for high-throughput studies to investigate its phenotype under more conditions

Aim of the experiment

- Replacing petrochemistry with bio-based processes for sustainable development
- Optimization of productivity, product yield and robustness requires modification in the configuration
- Swapping Embden-Meyerhoff-Parnas pathway of glycolysis in *Saccharomyces cerevisiae*
    - *12 reactions catalyzed by 26 cytosolic isoenzymes*
    - *genes encoding glycolysis are in 12 of 16 yeast chromosomes*

The experiment

A two-stepped approach:

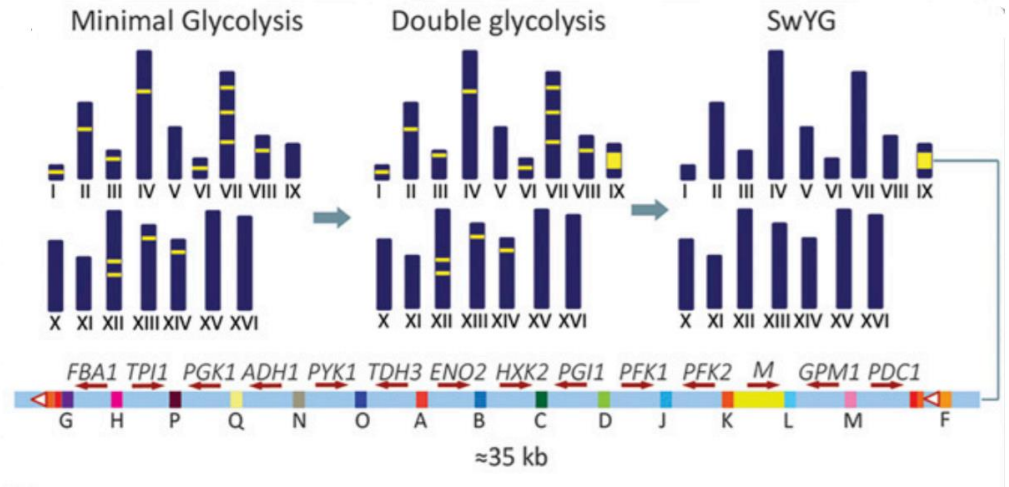
First step:

- reducing the complexity of yeast glycolysis
- deleting the structural genes for 13 of the 26 glycolytic enzymes

Second step:

- remaining 13 genes expressed from a single chromosomal locus
- remaining scattered native genes removed

→ Switchable yeast glycolysis (SwYG)

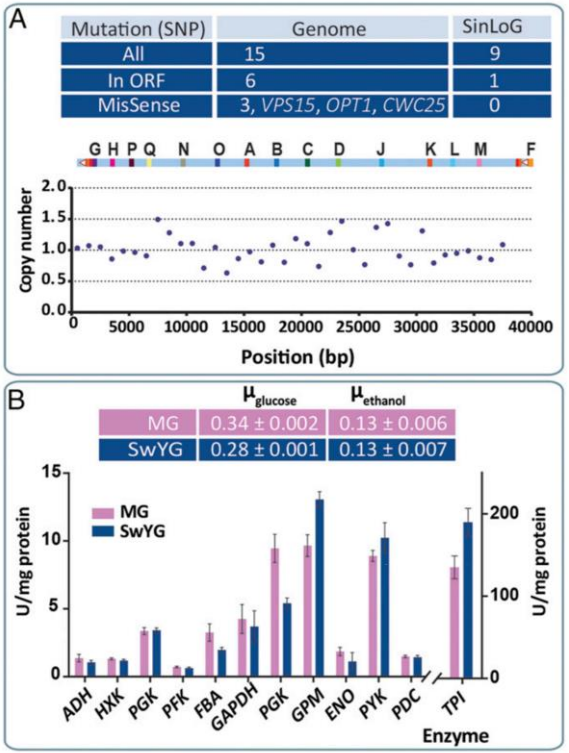


Engineering for a Yeast Platform for Glycolysis Swapping

Whole-genome sequence to confirm:

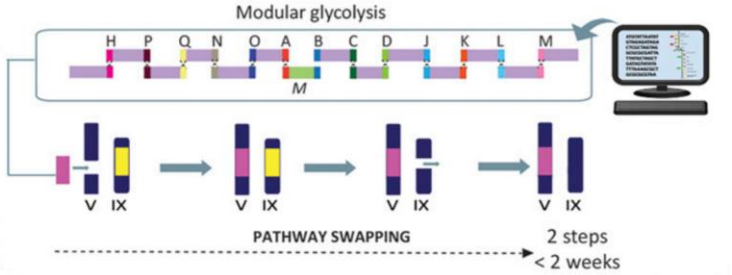
1. The correct sequence of single-locus native glycolysis
2. Deletion of the native glycolytic genes from their original loci
3. Absence of duplicated glycolytic genes in the single-locus native glycolysis and in the genome

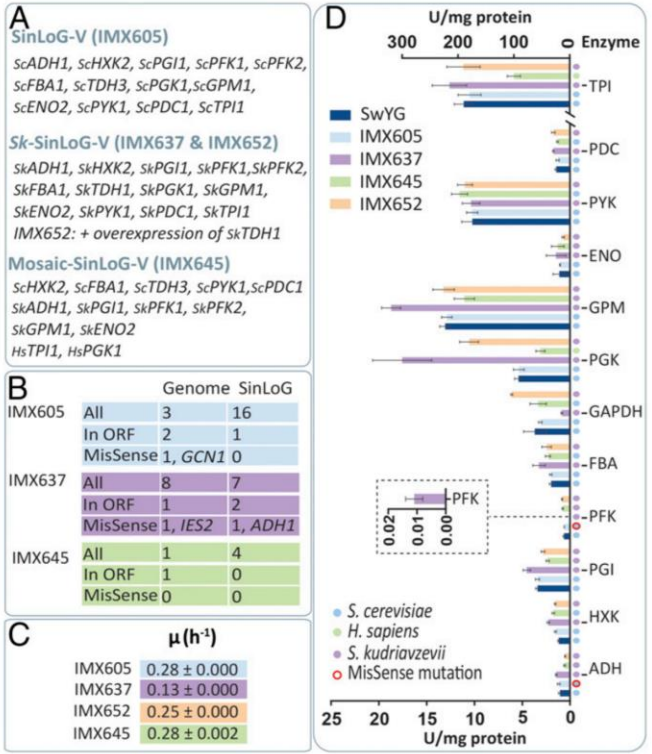
- Growth rate, glucose uptake and ethanol of the SwYG were decreased from the parental MG strain
- Biomass and product yields of glucose remained unaffected



Testing the feasibility

- Attempting to exchange SinLoG from chromosome IX to chromosome V
  - CRISPR/Cas9 targeting *CAN1* locus on chromosome V
  - excising the cluster from chromosome IX
- Confirmation of successful relocalization with whole-genome sequencing
- Similar growth rates and same activity of glycolytic enzymes in cell extracts





Discussion

- Pathway swapping enables the systematic analysis of of heterologous complementation of entire pathways
- Enables the humanization of different pathways
  - enables testing of the impact of mutations or drugs on human proteins
- Applications such as
  - functional analysis of heterologous proteins
  - testing kinetic models
  - exploring the effect of genomic location

References

Daniel Solis-Escalante, Niels G. A. Kuijpers, Nuria Barrajon-Simancas, Marcel van den Broek, Jack T. Pronk, Jean-Marc Daran, and Pascale Daran-Lapujade. **A Minimal Set of Glycolytic Genes Reveals Strong Redundancies in *Saccharomyces cerevisiae* Central Metabolism.** Eukaryot Cell. 2015 Aug; 14(8): 804–816. doi: 10.1128/EC.00064-15.

Niels G. A. Kuijpers, Daniel Solis-Escalante, Marijke A. H. Luttik, Markus M. M. Bisschops, Francine J. Boonekamp, Marcel van den Broek, Jack T. Pronk, Jean-Marc Daran, Pascale Daran-Lapujade **Pathway swapping: Toward modular engineering of essential cellular processes.** Proc Natl Acad Sci U S A. 2016 Dec 27; 113(52): 15060–15065. doi: 10.1073/pnas.1606701113