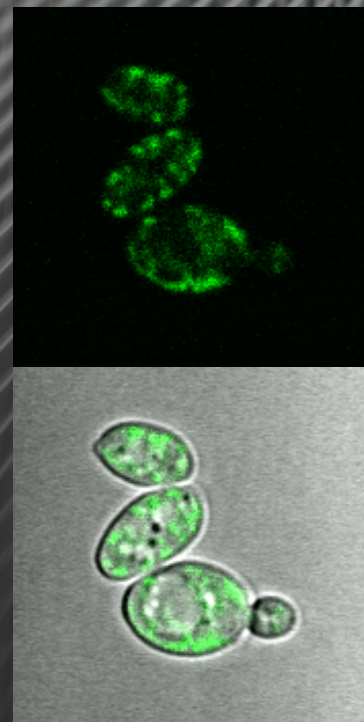


Exploring cell structure and metabolism of *Yarrowia lipolytica*

February 19, 2020

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Pacific Northwest National Laboratory



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- Environmental Molecular Sciences Laboratory

- Team of teams

- Omics
- Bioimaging
- Cellular Dynamics

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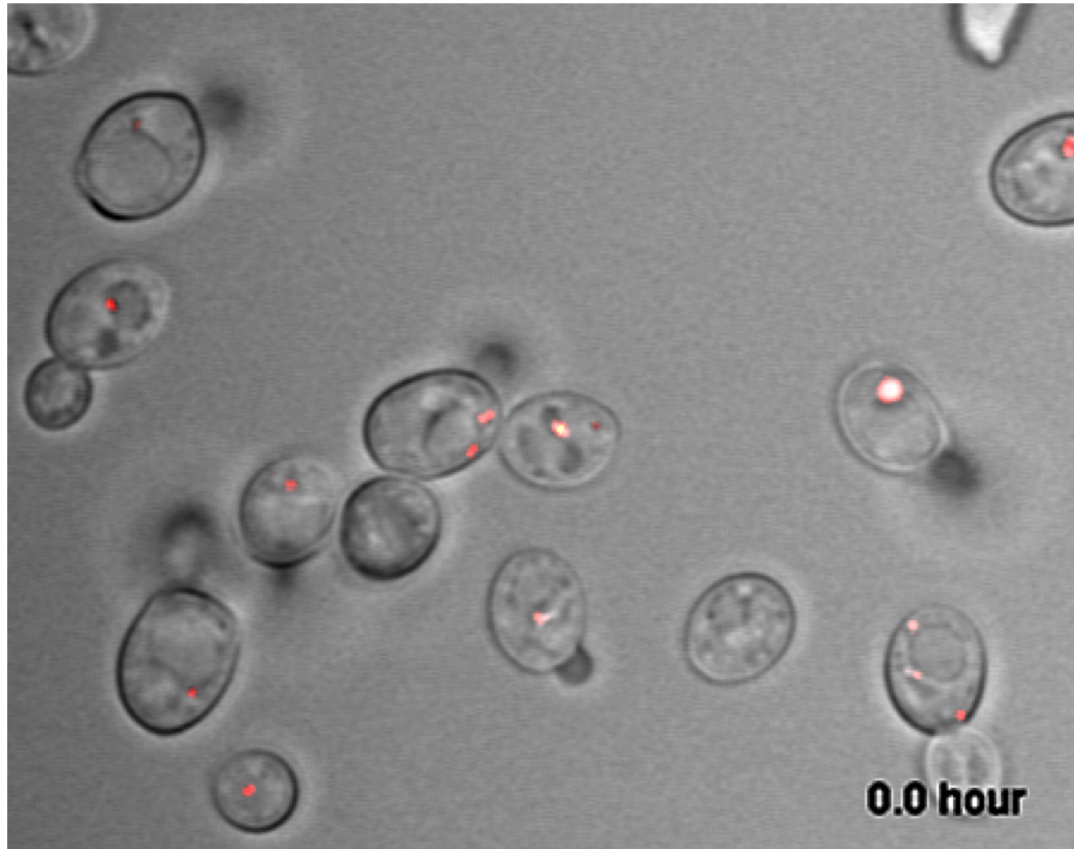
Neeraj Kumar

others former and current...



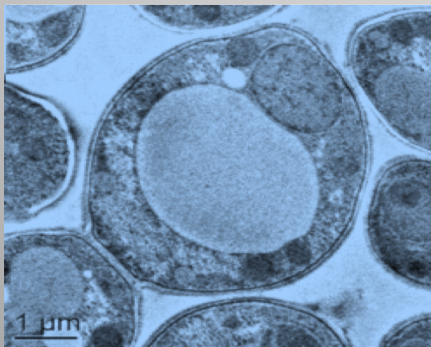
Yarrowia lipolytica, a model oleaginous yeast

Response to nitrogen limitation



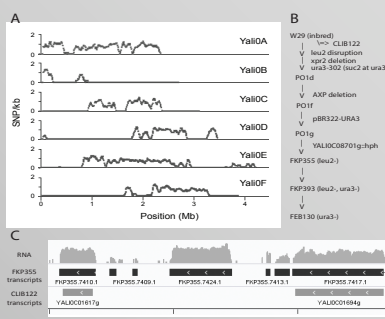
- Sequenced genome
- Dimorphic
- Molecular genetics
- Metabolic model

We are applying a variety of tools for characterizing phenotypes



Visualization of ultra-structures and whole cell reconstruction: TEM

Genetics (ku strain development) and genomics

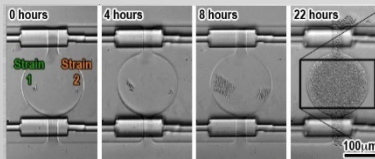


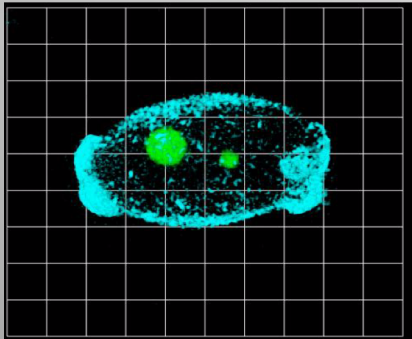


Model pathways: Modeling & simulation



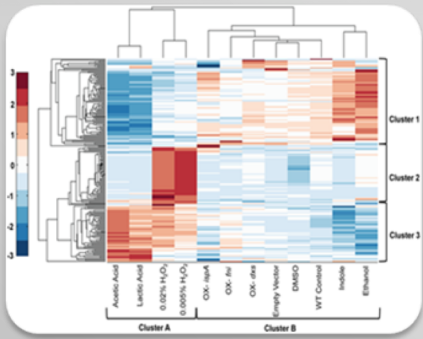
Microfluidic for single cell capture





Visualization of biological compartments

Decipher metabolic networks and enable genetic engineering approaches: integrated omics

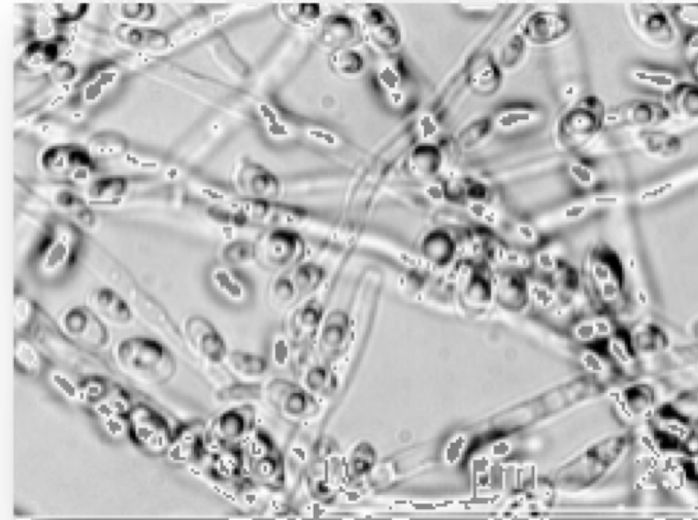


Yarrowia lipolytica makes hyphae in bioreactors

MTYL038



MTYL053



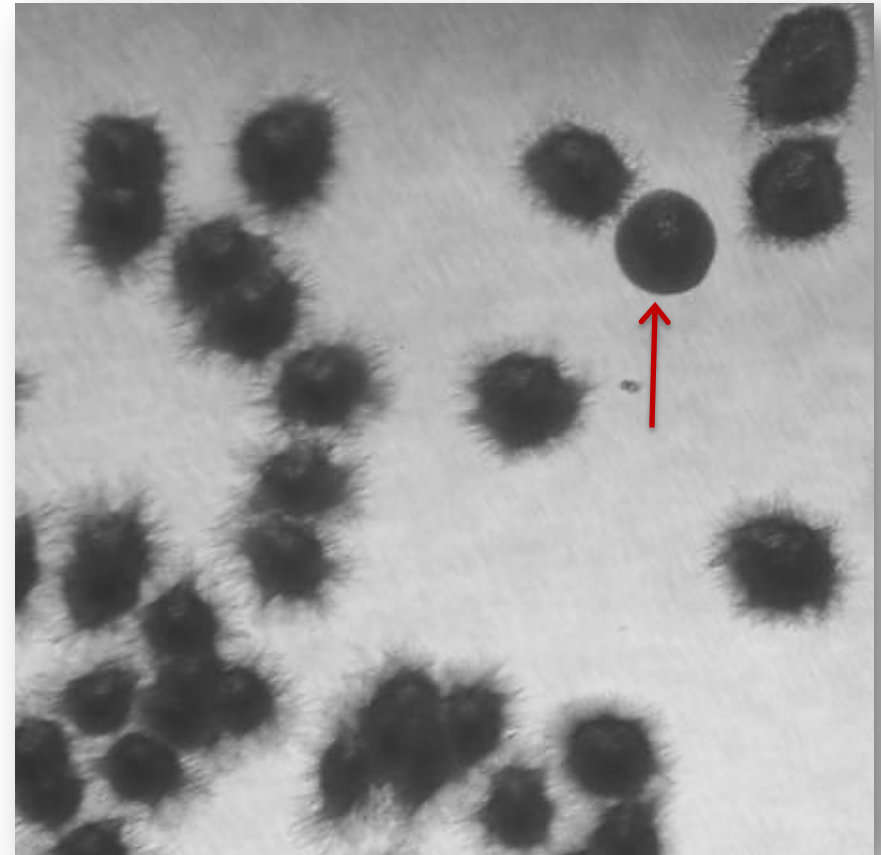
Images courtesy of Ed Kerkhoven, Chalmers

- Hyphae clog inlet and outlet ports, tubing and filters
- Hyphae tend to form chunks and reduce homogeneity
- Multiple growth modes make growth rate control difficult
- Experimental reproducibility goes down

Smooth mutants do not make hyphae

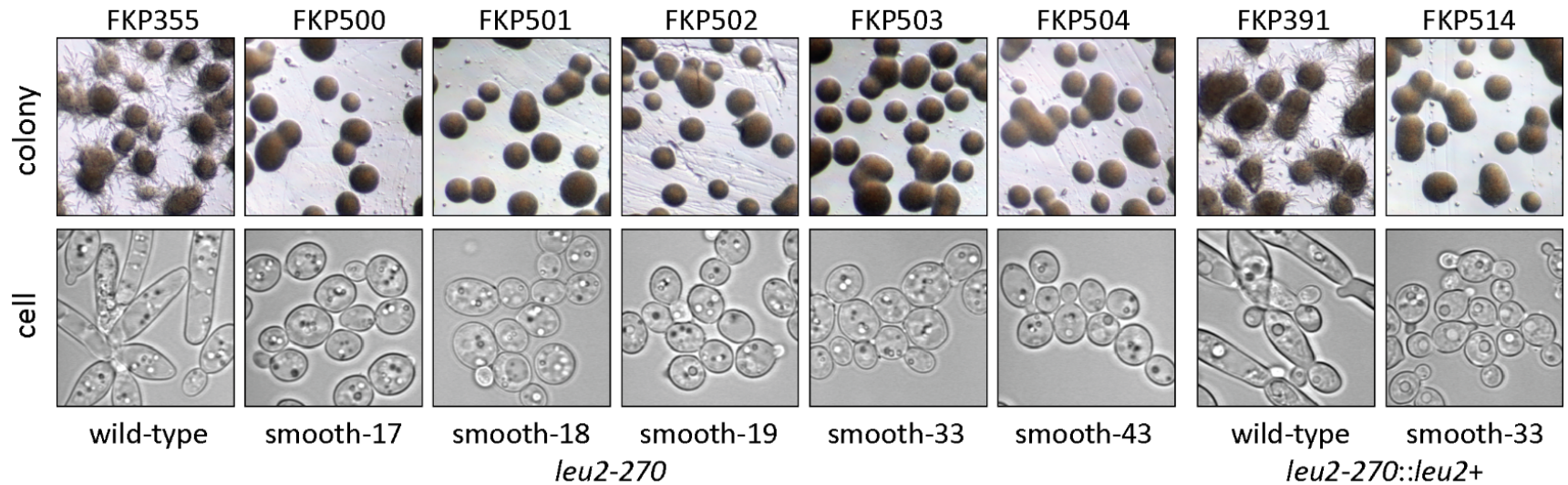
- Screened ~500,000 colonies for lack of hyphae
- Isolated 65 mutants
- Confirmed that 5/65 mutants (smth 1-5) don't make hyphae under any tested environmental conditions

Select mutants that form smooth colonies



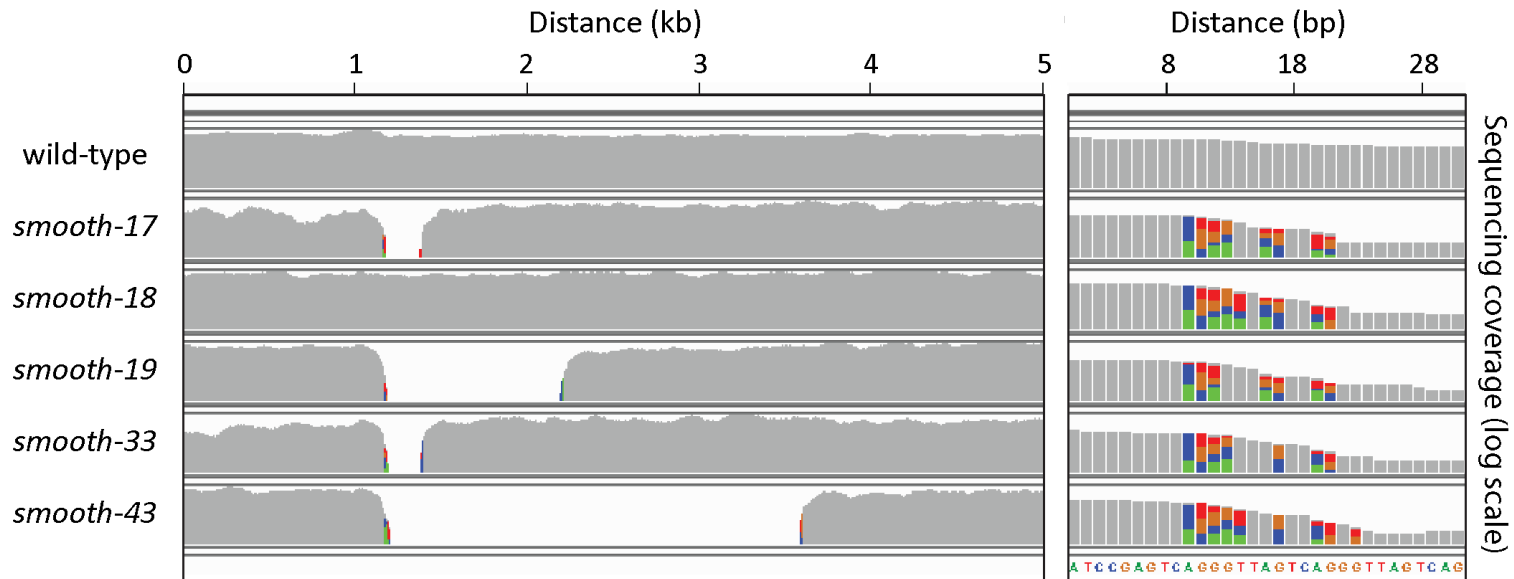
colony morphology, 80x

Screening for *smooth* strains



Approximately 500,000 colonies were screened for smooth morphology with no visible hyphae

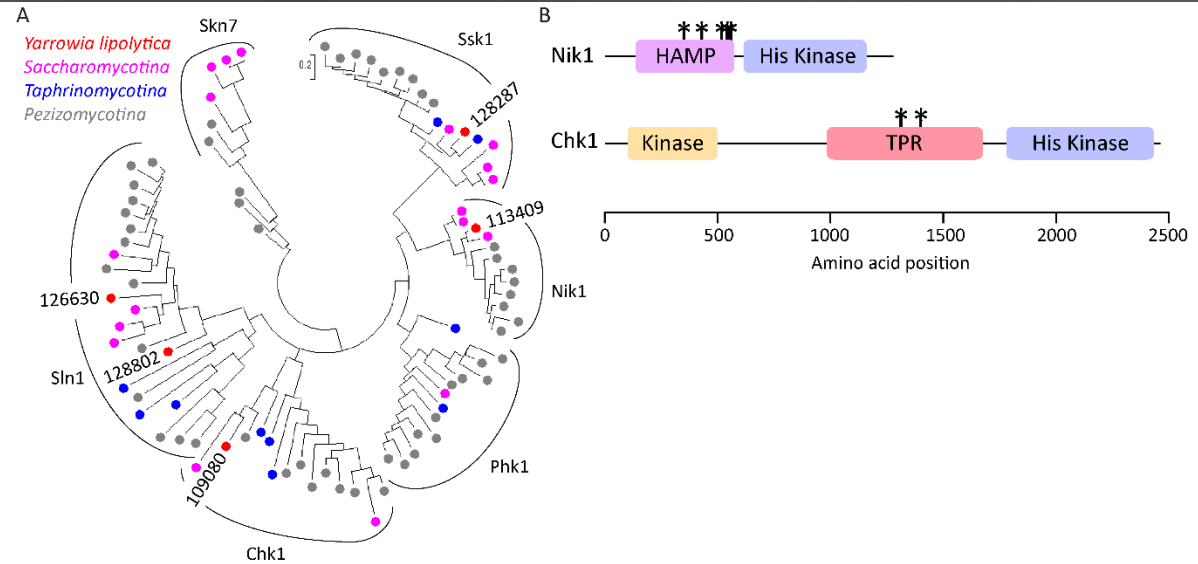
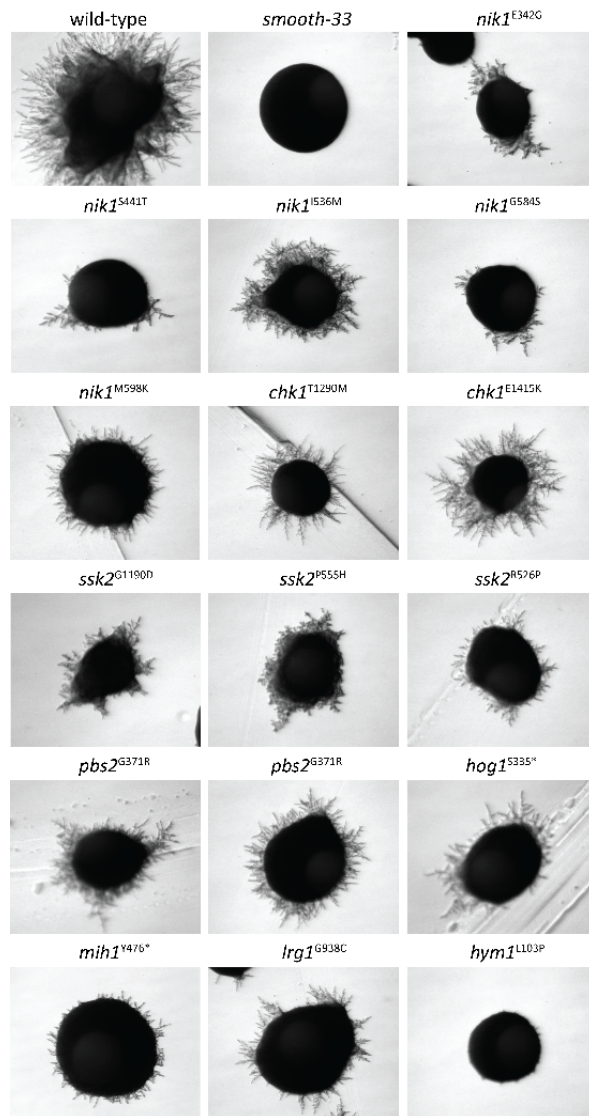
Resequencing *smooth* strains with PacBio



Raw PacBio reads with homology to the single-copy region at the end of scaffold 14 (from 1-12 kb) were re-assembled and analyzed



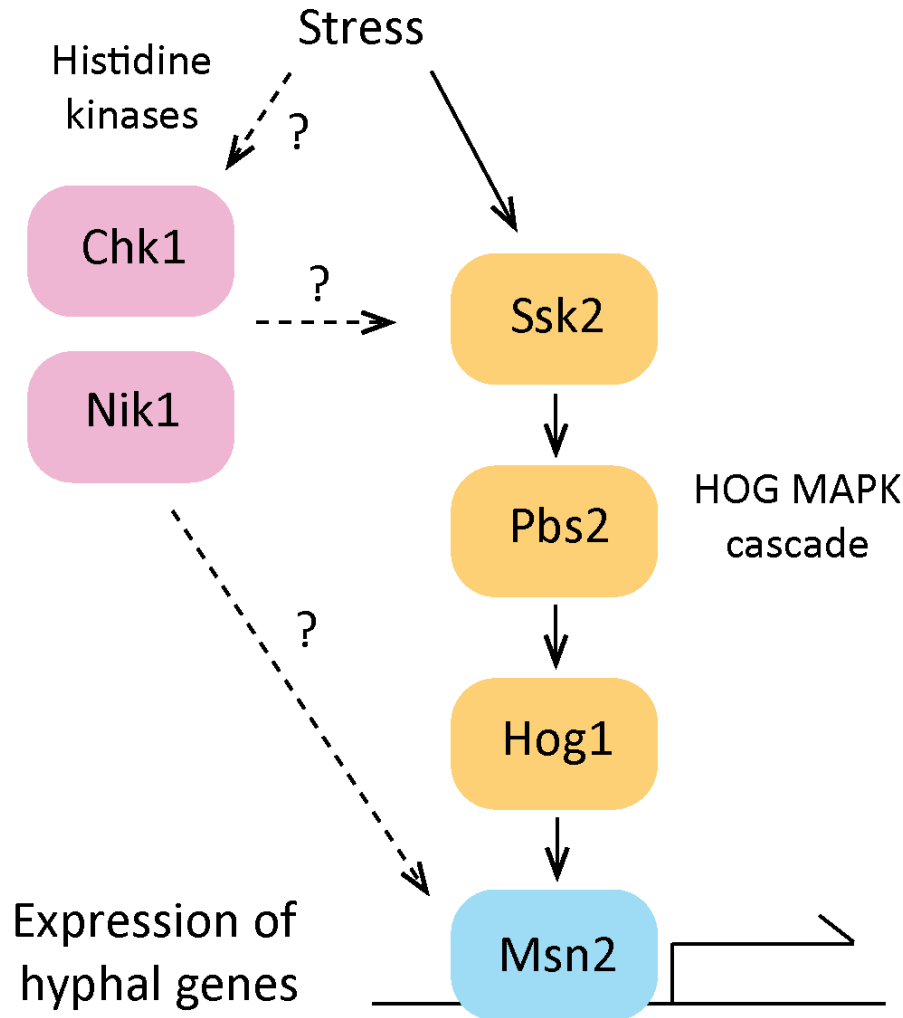
Suppressor screen and resequencing



► Mutations in stress signaling pathways

- MAP kinase
- Histidine kinase

Summary

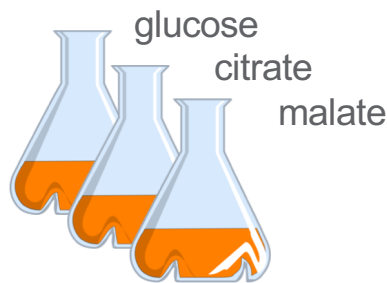


- ▶ Generated non-hyphal *Yarrowia* mutants
- ▶ Resequenced *smooth* mutants and found mutations in repeat regions
- ▶ Suppressors of *smooth* involved in signaling

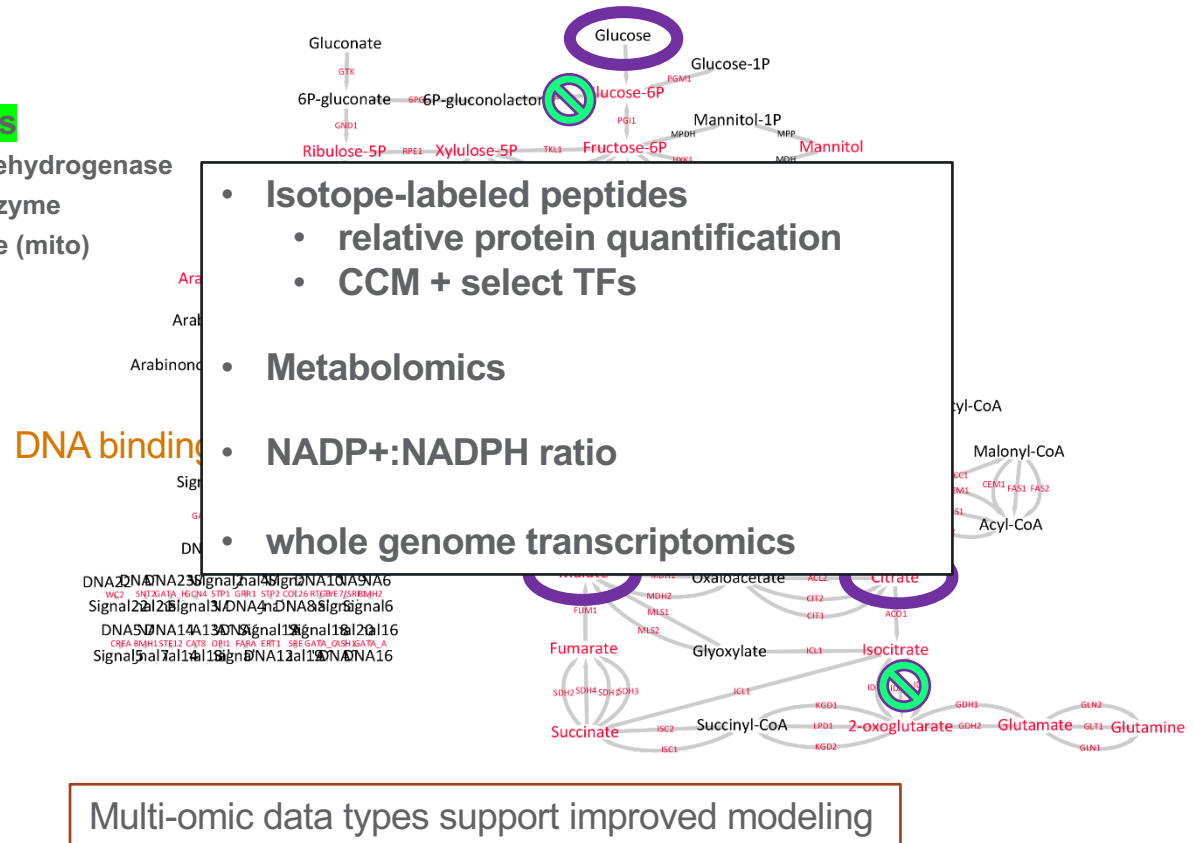
Experiment: proteins and metabolites of CCM by Single Reaction Monitoring (SRM)

- wild-type
- 3 NADPH regeneration mutants
 - ZWF1: glucose-6 phosphate dehydrogenase
 - MAE1: mitochondrial malic enzyme
 - IDP1: isocitrate dehydrogenase (mito)

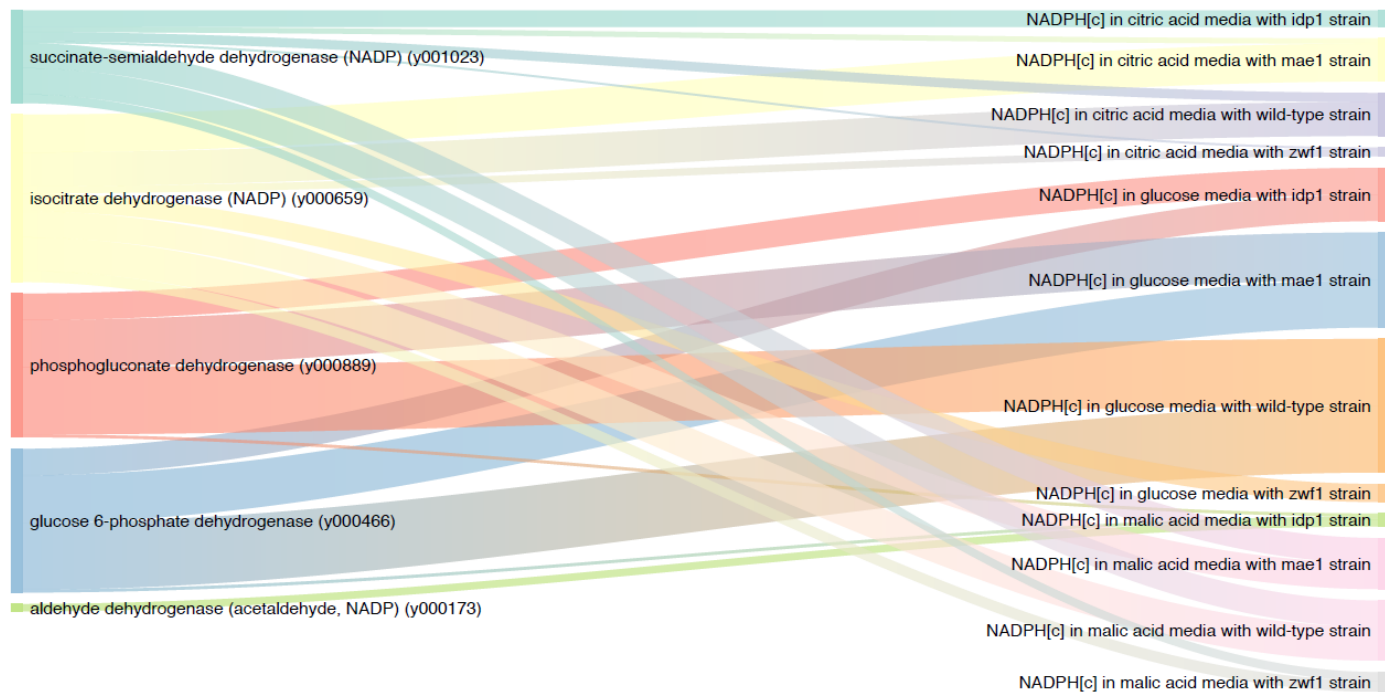
- 3 carbon sources



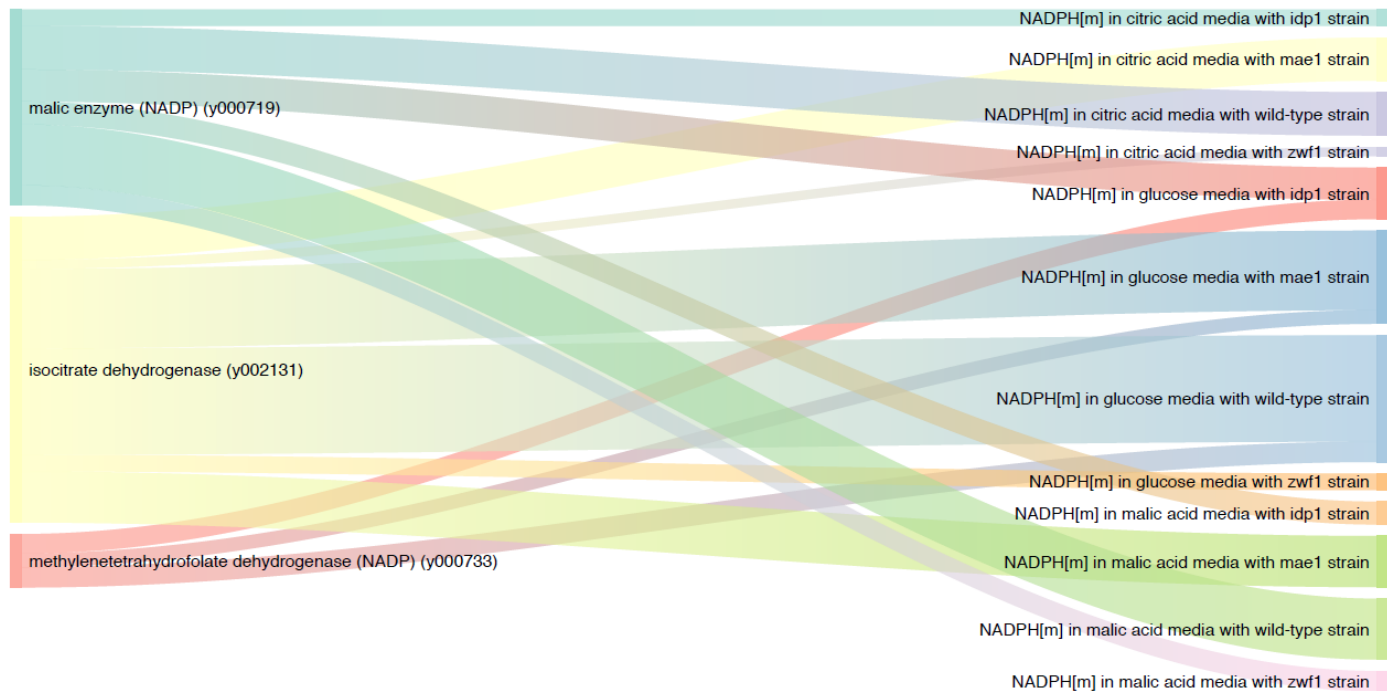
4 strains x 3 conditions
triplicates



Data to model: NADPH generating fluxes - cytoplasmic

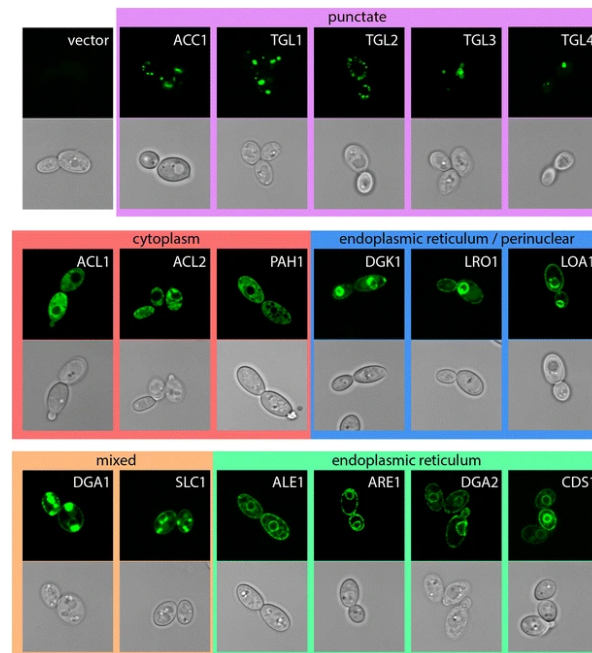


Data to model: NADPH generating fluxes - mitochondrial



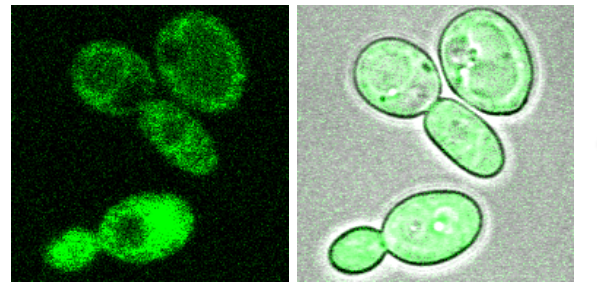
Yarrowia lipolytica Cell Atlas

under construction	compartment
aca1	mitochondrion
aca2	peroxisome
lat1	mitochondrion
fas1	cytosol
fas2	cytosol
cem1	mitochondrion
cit1	mitochondrion
cit2	peroxisomal
mdh1	mitochondrion
mdh2	cytosol/peroxisome
transcription factors	
creA	nucleus
col26	nucleus
far1	nucleus
rtg3	nucleus
gzf1	nucleus
stp2	nucleus
ert1	nucleus
sre1	nucleus

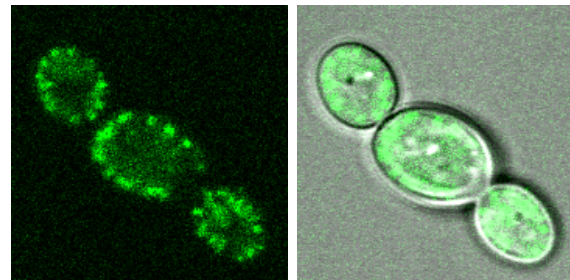


lipid biogenesis	compartment
acc1	LD
slc1	ER and LD
dga1	LD
gpt2	ER
carbon use	
zwf1	cytosol
idp1	mitochondrion
mae1	mitochondrion
lipolysis/autophagy	
atg8	cytosol/vacuole
pex10	peroxisome
nitrogen recycling	
arg4	cytosol
arg5	cytosol
car1	plasma membrane
put2	mitochondrion

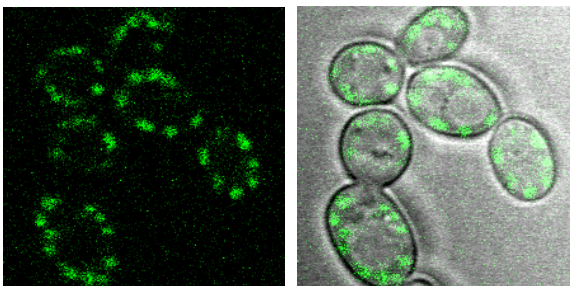
Yarrowia lipolytica Cell Atlas



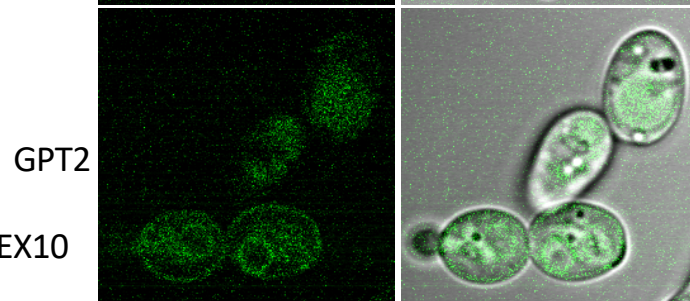
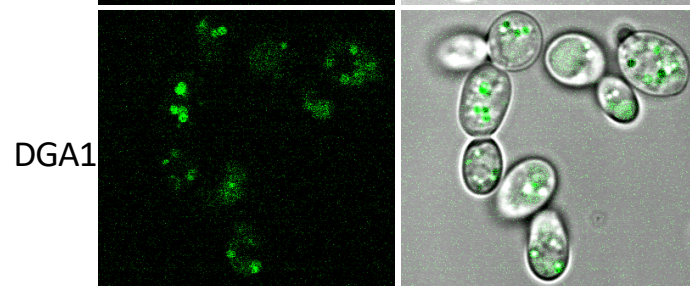
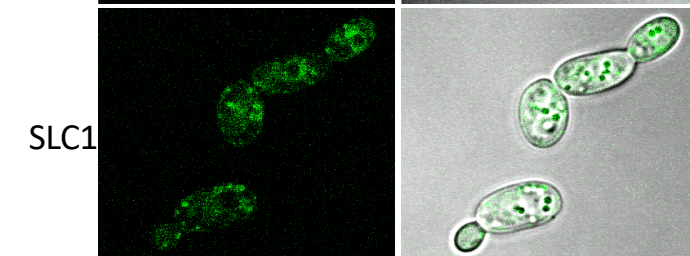
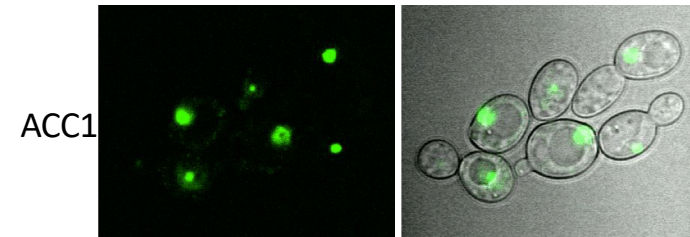
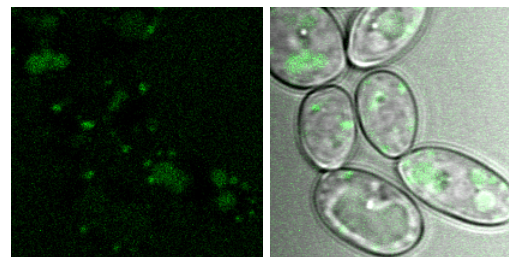
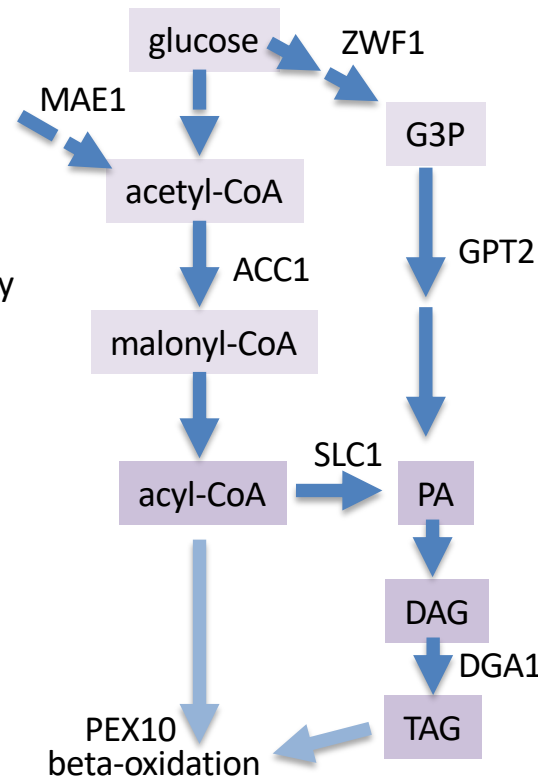
ZWF1 pentose phosphate pathway



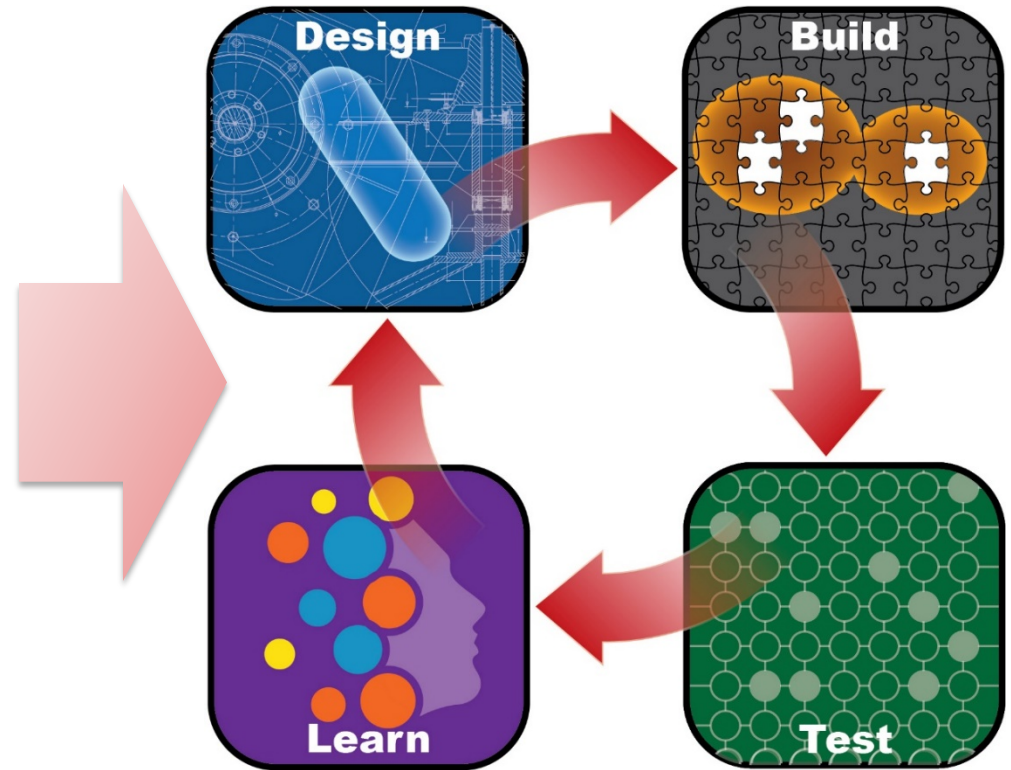
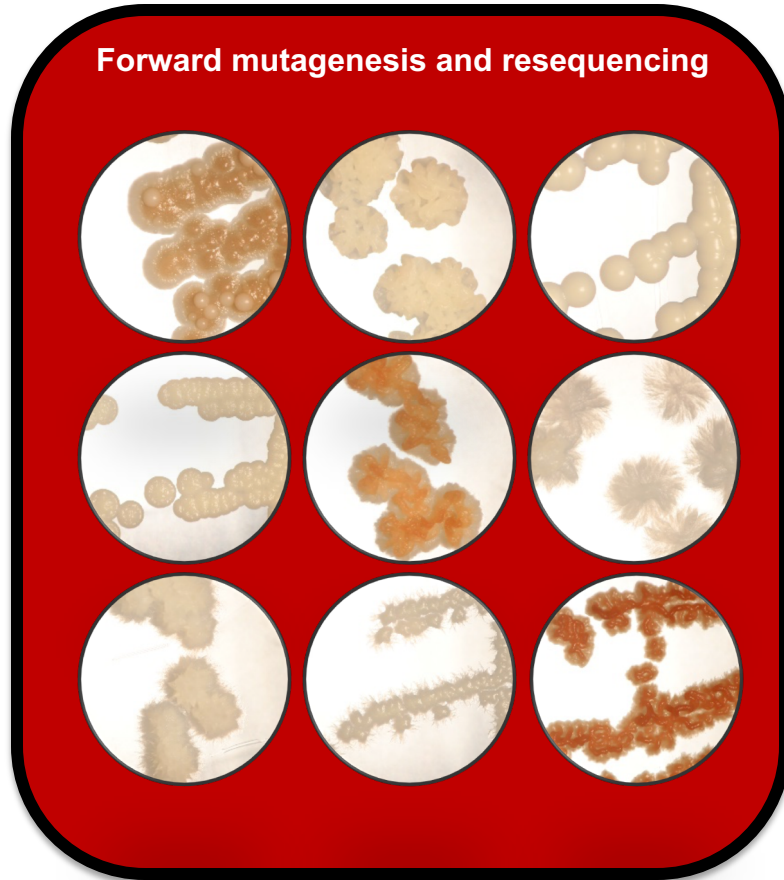
MAE1 malate synthase



PUT2 glutamate recycling



Future directions: Synthetic biology and fast forward genetics in *Yarrowia lipolytica*



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