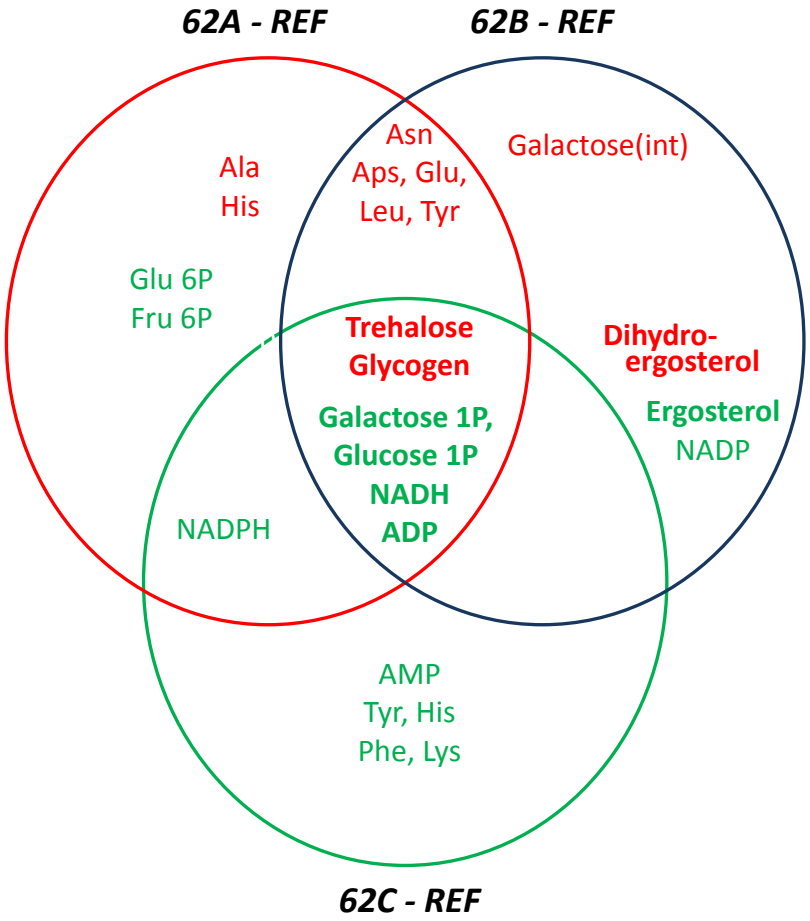
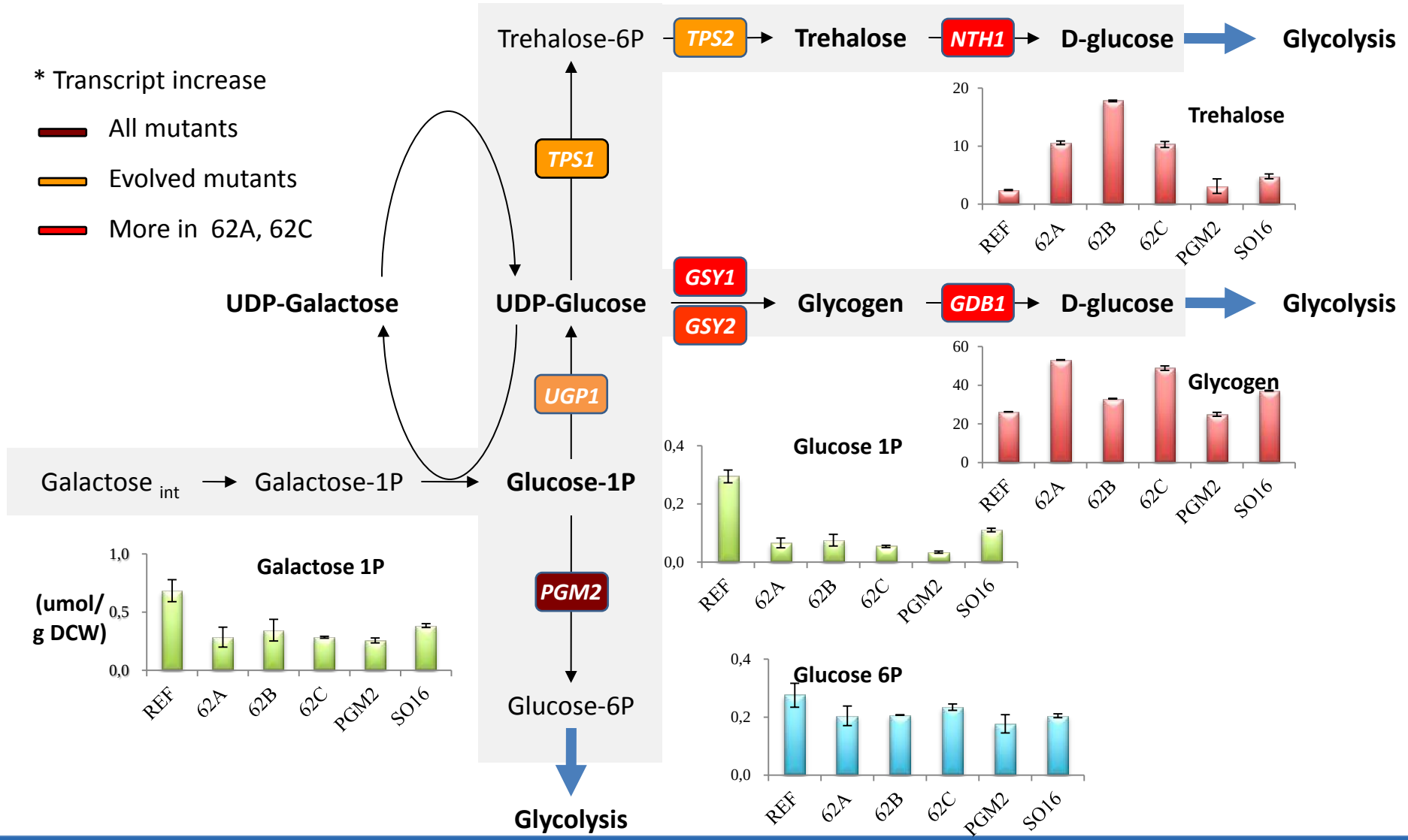
Galactose utilization

Metabolome analysis



Galactose utilization

Integration of transcriptome and metabolome



Galactose utilization

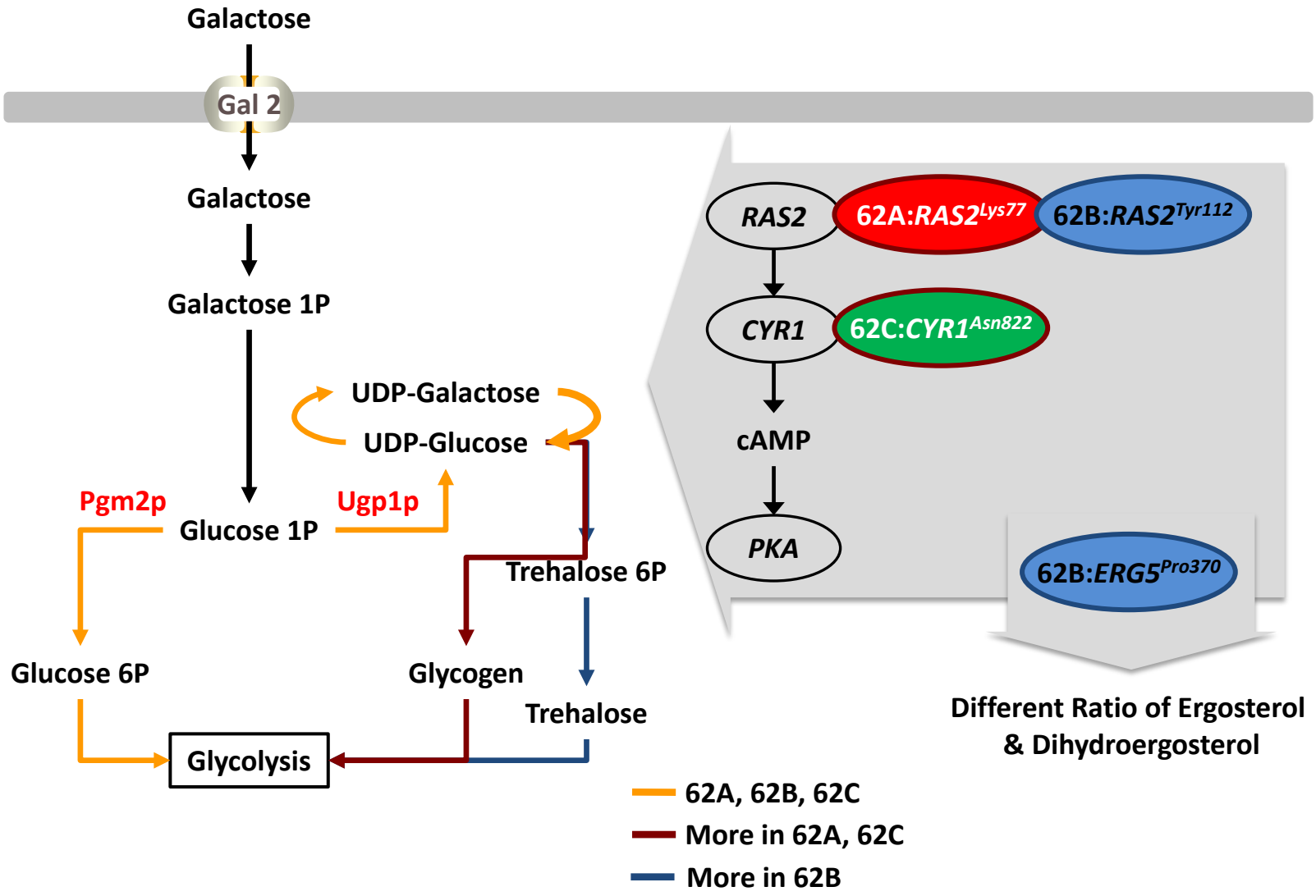
Genome analysis

No mutations in *GAL* genes, including *PGM2* and genes associated with storage carbohydrate metabolism (both coding and non-coding regions)

→ Adaptation strategies different from rational engineering
→ What are the **"driving mutations"** (beneficial mutations for galactose utilization)?

Galactose utilization

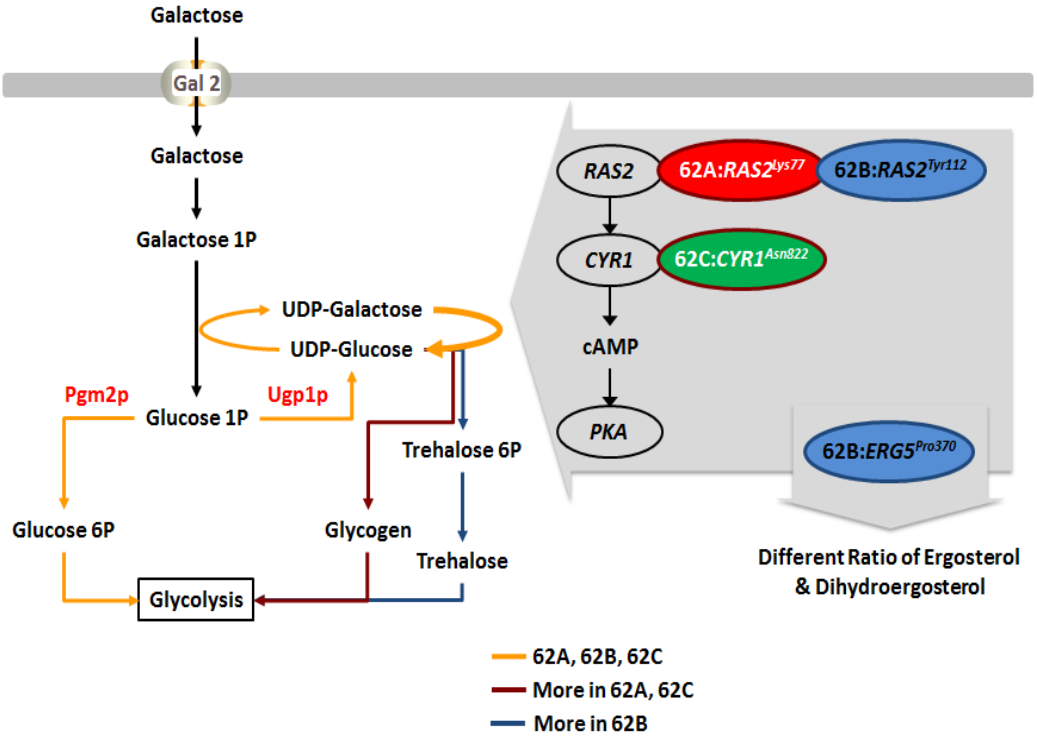
Hypothesis



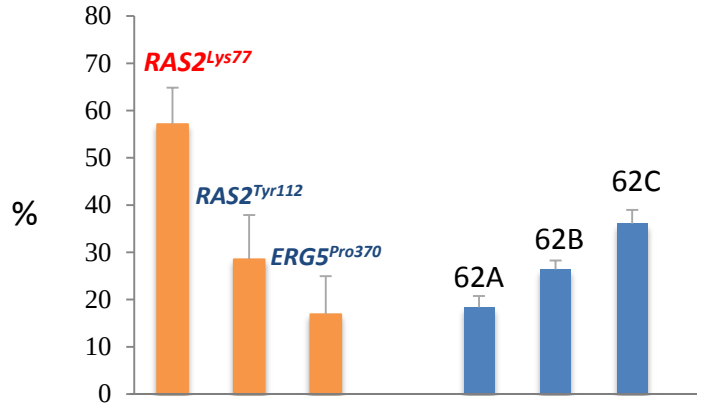
Galactose utilization

Inverse metabolic engineering

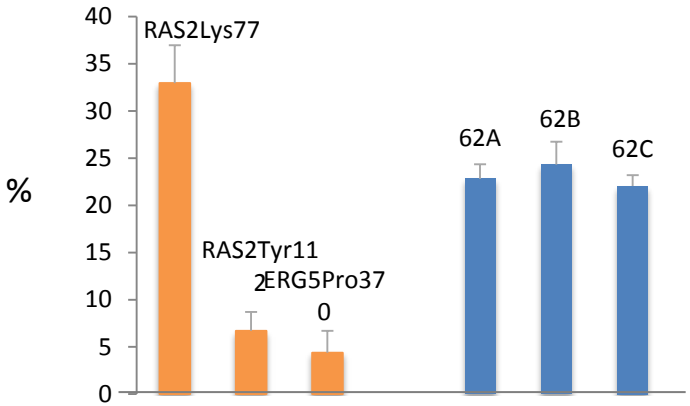
Evaluation of two single nucleotide mutations identified in *RAS2*
and one single nucleotide mutation in *ERG5*



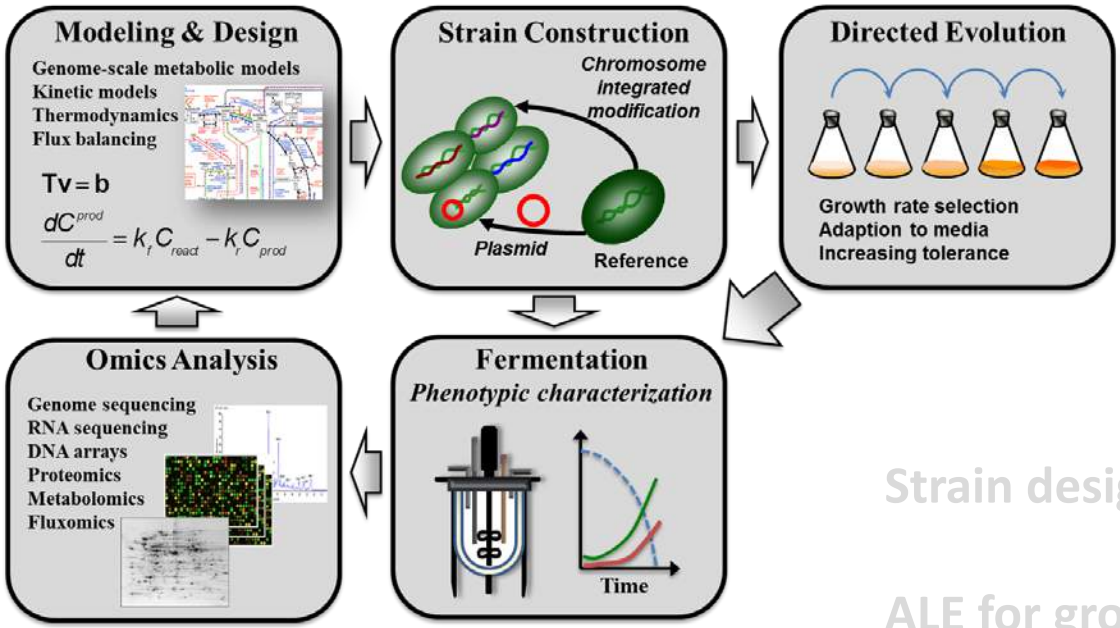
Improvement of specific galactose uptake rate (%)



Improvement of maximum specific growth rate (%)



Outline



Strain design for succinic acid production

ALE for growth in galactose

ALE for temperature tolerance

Temperature tolerance

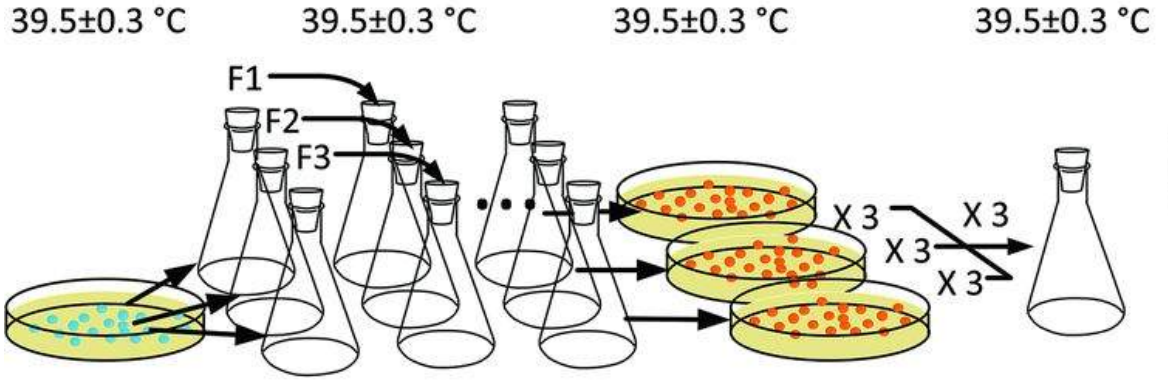
Enzymes for biomass degradation often derived from thermophilic organisms
-> activity at high temperature

$\Rightarrow$ Thermotolerant yeast strains needed for simultaneous saccharification and fermentation

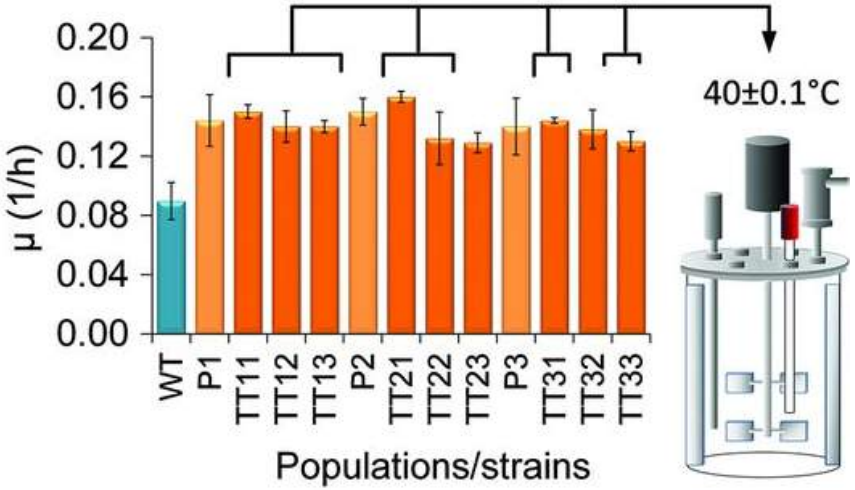
Fermenter cooling is expensive

Temperature tolerance

Evolution



Characterization



Temperature tolerance

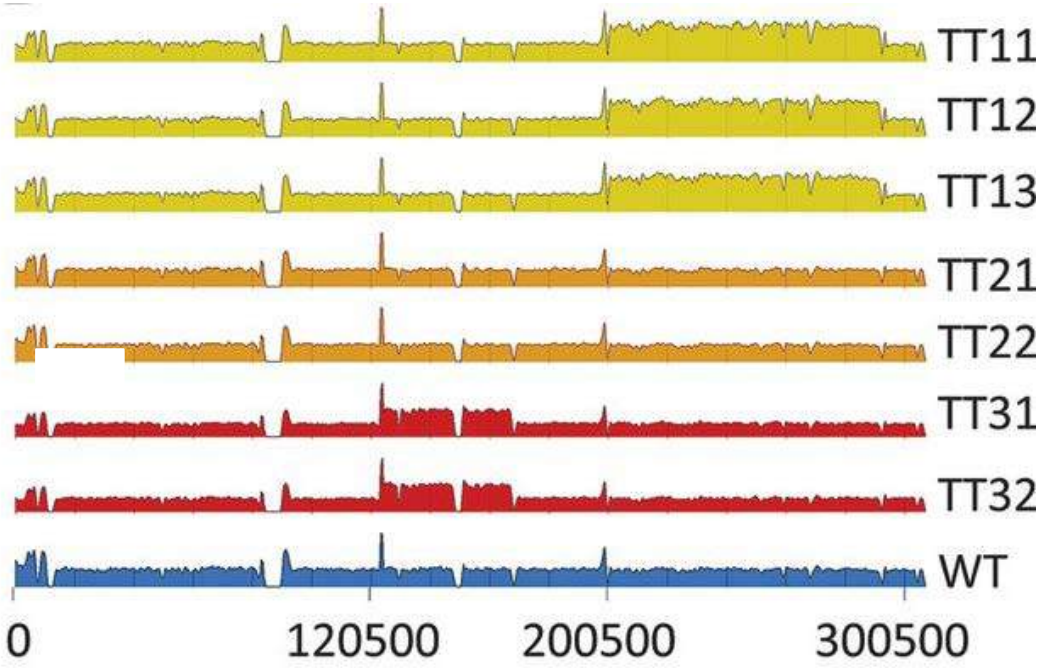
Characterization of thermotolerant strains

- higher growth rate at 40°C
- higher biomass yield
- higher ethanol production
- no growth on non-fermentable carbon sources
- higher tolerance towards osmotic stress
- reduced tolerance towards oxidative stress

Temperature tolerance

Genome sequencing

⇒ partial duplications of Chr III



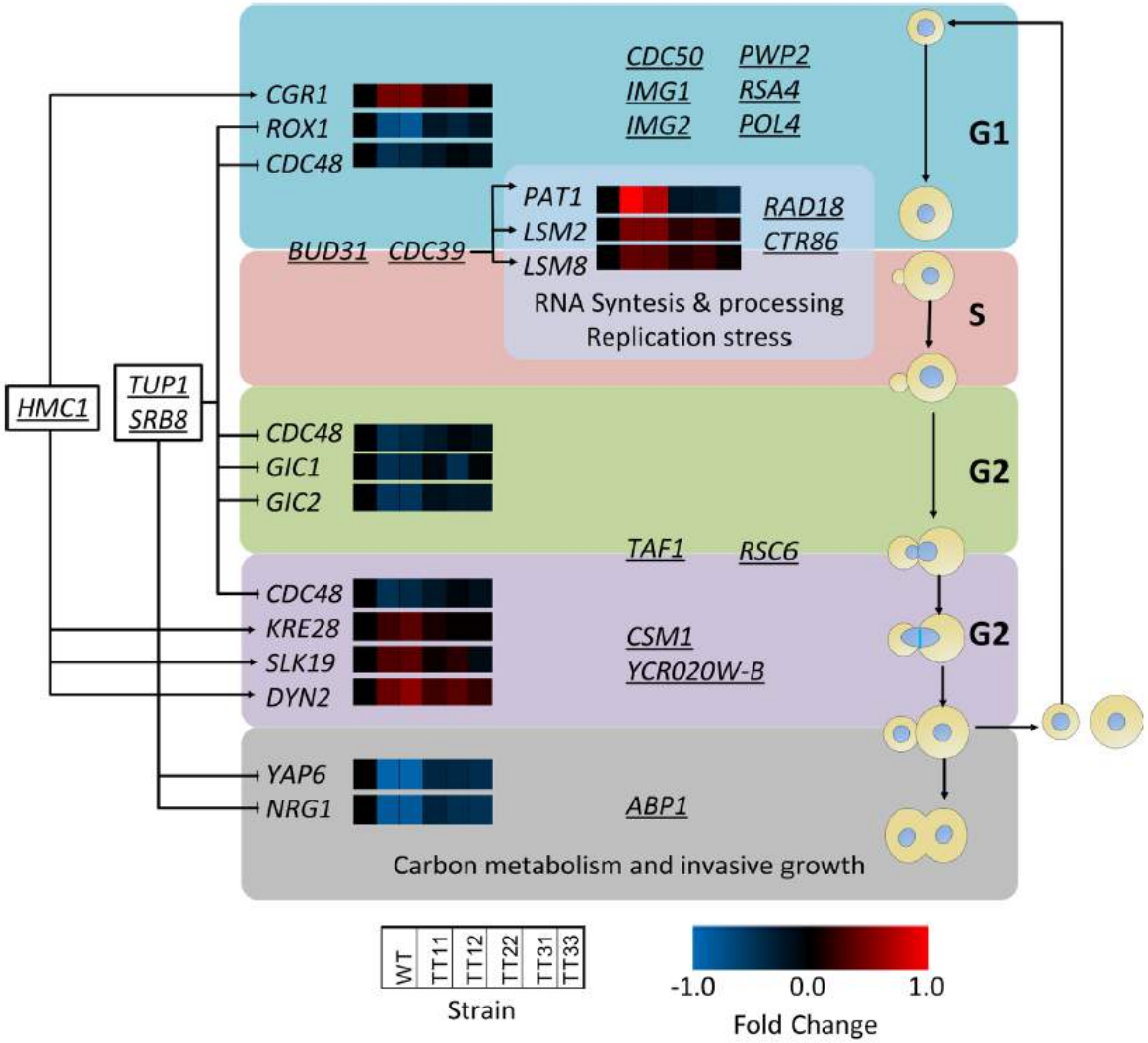
Caspeta *et al.* (2014) Science 346:75-78

Temperature tolerance

Dublication of several genes involved in the cell cycle (underlined)

including transcription factors that regulate cell cycle genes

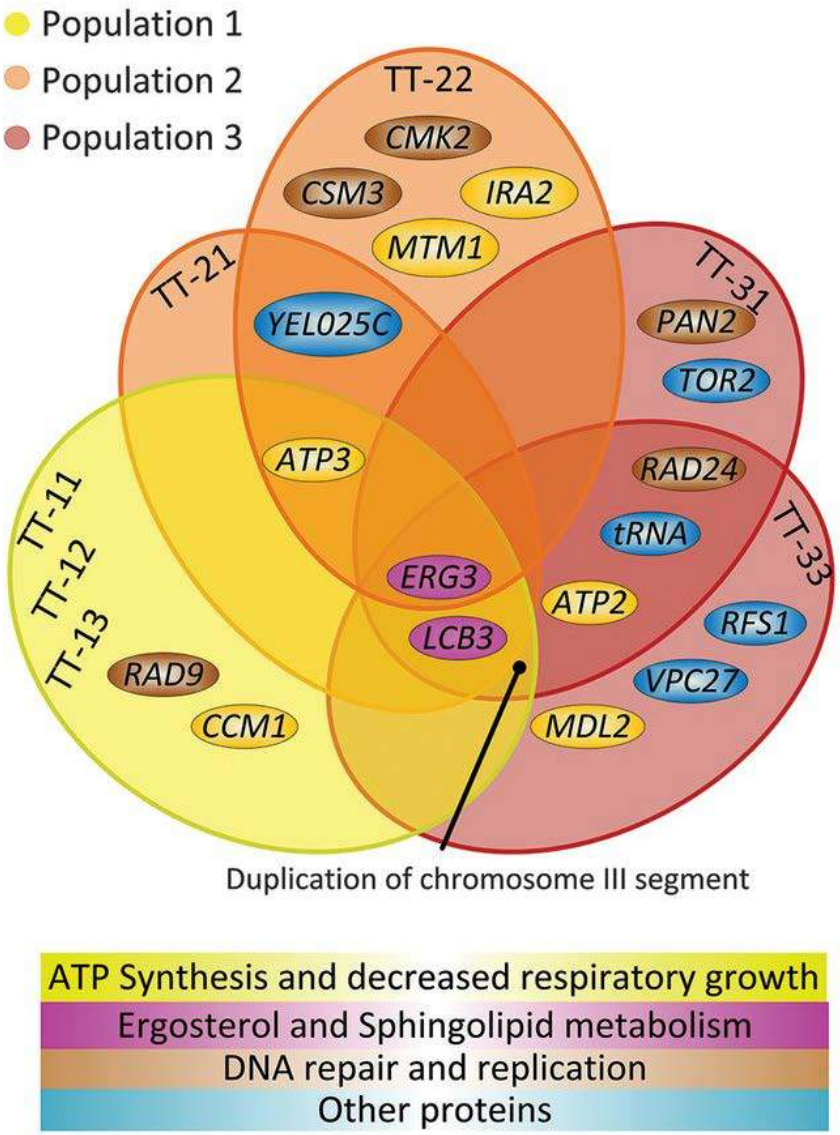
=> Chromosome duplication as fast adaptation mechanism



Temperature tolerance

Genome sequencing

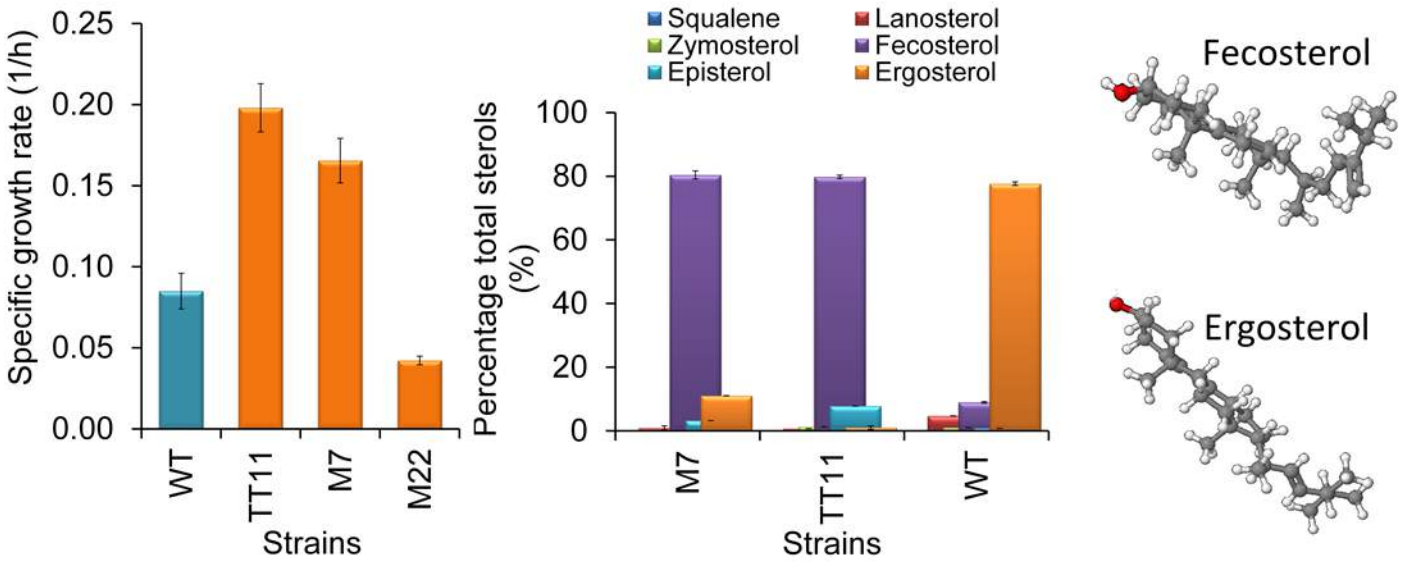
=> point mutations



Temperature tolerance

Evaluation of point mutations

A large fraction of the SNVs results in appearance of stop codons (26%), and 66% of these are in *ATP3* and *ERG3*



Introduction of one of the stop codons identified in *ERG3* results in 85% recovery of the temperature tolerance

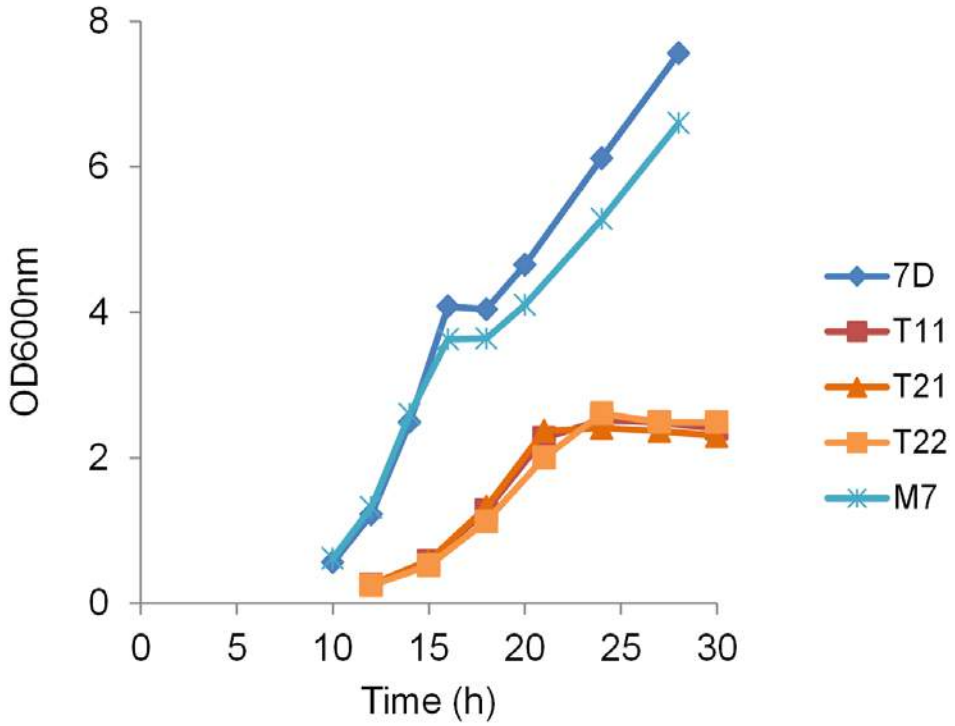
and

results in the same remodeling of the sterol composition as found in the TT strains

Temperature tolerance

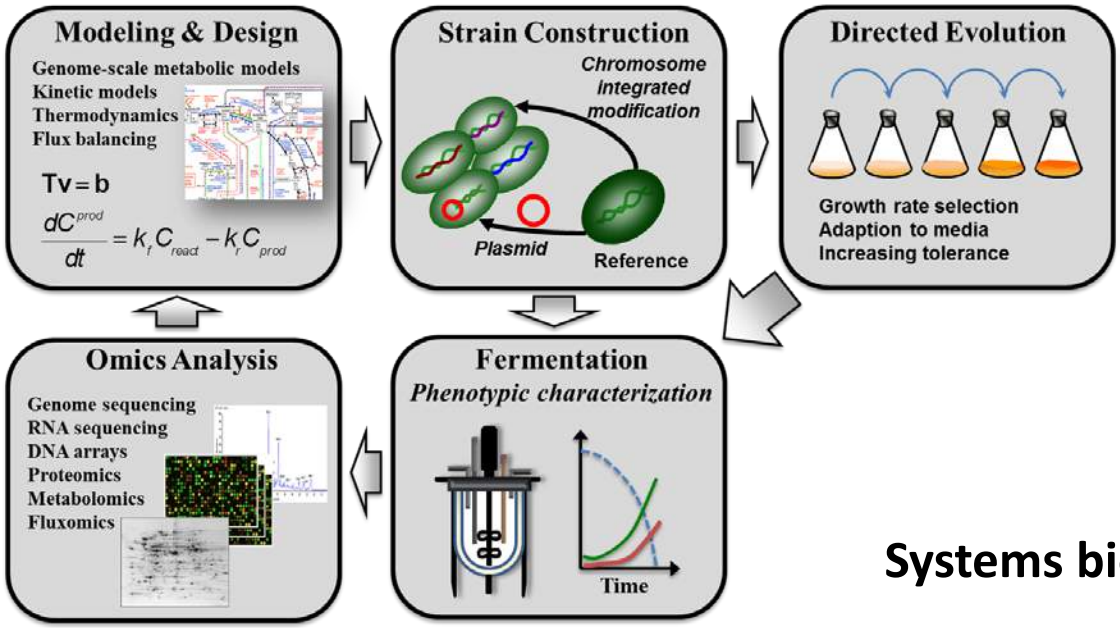
Thermotolerant strains showed trade-off at 30°C

The M7 strain with re-introduced point mutation does not show any trade-off at 30°C and grows on non-fermentable carbon sources



Caspeta *et al.* (2014) Science 346:75-78

Conclusion



Systems biology can help in

- **Predicting engineering targets**
- **Explaining adaptation mechanisms**

Thank you