CHALMERS UNIVERSITY OF TECHNOLOGY

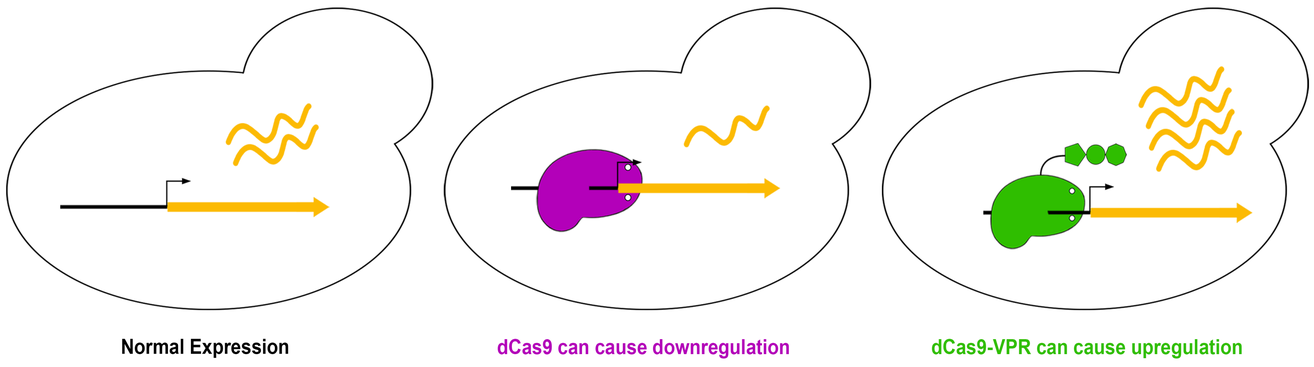
INDUSTRIAL BIOTECHNOLOGY

Fine-Tuning the Stress Response of *Saccharomyces cerevisiae* using CRISPR interference Technology

Luca Torello Pianale, Vaskar Mukherjee, Elena Camara and Yvonne Nygård

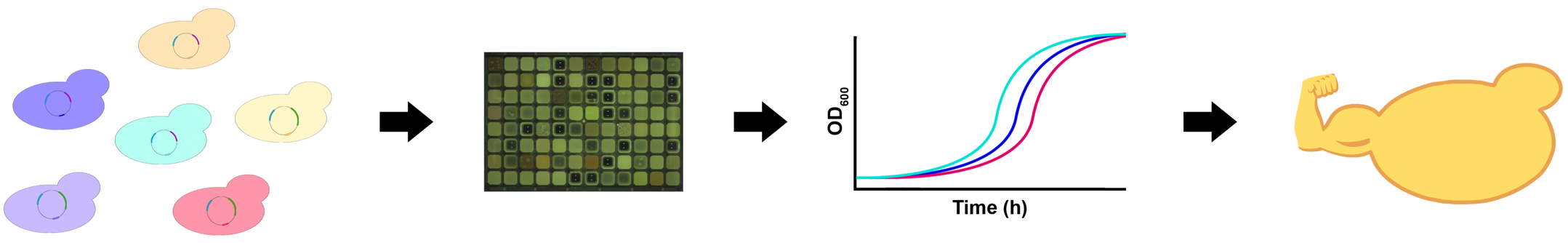
Division of Industrial Biotechnology, Department of Biology and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

CRISPRi Technology



- The CRISPRi technology can alter gene expression without permanent genetic alterations.
- A catalytically inactive Cas9 protein (dCas9) fused to a repressor or an activator (such as VPR, a combination of 3 activation domains) can be targeted to a genetic locus using an sgRNA.
- dCas9 bound to the DNA can cause steric encumbrance, leading to gene repression, while dCas9 alone or fused to activation domains can lead to enhanced transcription activation.

Workflow



Generation of CRISPRi mutants with a high-throughput method developed in our lab.

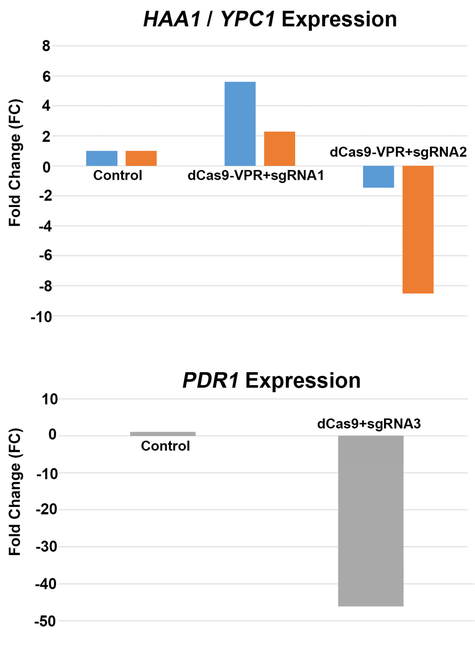
High-throughput screenings with 96-well plates in Growth Profiler 960 (Enzyscreen).

Generation of growth curves and growth parameters were extracted from the raw data.

Identification of yeast strains that are more robust towards lignocellulosic inhibitors.

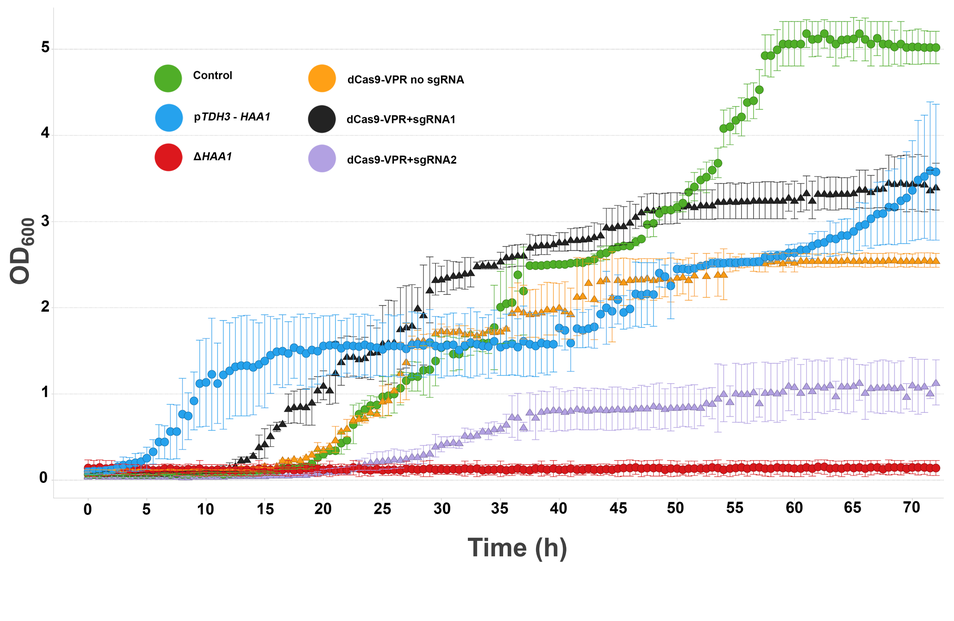
Screening, 125 mM Acetic Acid

qPCR



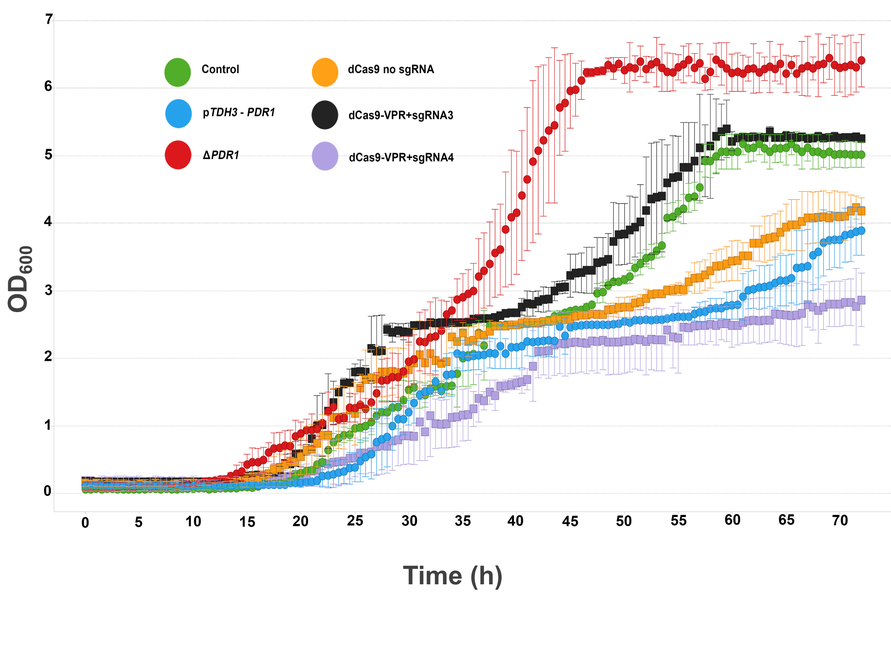
- Relative expression of *HAA1* (blue), *YPC1* (orange) and *PDR1* (grey), after 5 hours.
- The expression of *YPC1* is regulated by *HAA1.* Expression is normalized to *ACT1*.

HAA1



- Upregulation of *HAA1*, by dCas9-VPR+sgRNA1, targeting a region ~500 nt before the TSS of *HAA1*, decreased the lag phase in presence of acetic acid.
- Downregulation of *HAA1,* by dCas9-VPR+sgRNA2, targeting a region ~100 nt before the TSS of *HAA1,* drastically decreased tolerance to acetic acid.
- Expression of dCas9-VPR alone leads to a growth defect.

PDR1



- Down-regulation of *PDR1* by Cas9+sgRNA3 and *PDR1* disruption gave a slight increased robustness towards acetic acid.
- Targeting *PDR1* with dCas9+sgRNA4 at ~150 nt before the TSS gave a slight decrease in tolerance towards acetic acid.
- Expression of dCas9 alone leads to a growth defect.

Conclusions

1. Modification of the native regulation of *HAA1* and *PDR1* with the CRISPRi technology can confer fitness benefits under acetic acid stress.
2. CRISPRi is a highly potential technique to study the stress regulation in yeast, as this technology is less disruptive compared to traditional disruption and overexpression methods.

Vaskar Mukherjee
Vaskar@chalmers.se
Yvonne Nygård´s Lab

CHALMERS UNIVERSITY OF TECHNOLOGY
SE-412 96 Gothenburg, Sweden
www.chalmers.se

SPONSORED BY:

Swedish Energy Agency

indbio