Getting started: Adaptation of wine yeast to early fermentation conditions

George van der Merwe
Associated Professor

CCOVI: February 13th, 2013

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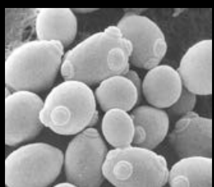
MOLECULAR and Cellular Biology

Research: van der Merwe lab



Wine production

- **Wine yeast development**
  - Molecular response to wine fermentation
  - Molecular response to Icewine fermentation
  - Molecular response to sparkling wine (secondary) fermentation
- **Microbial wine spoilage**
  - *Brettanomyces*

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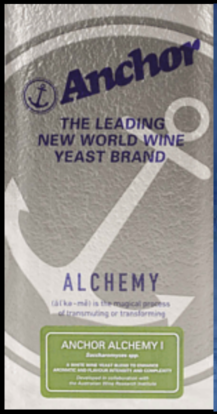
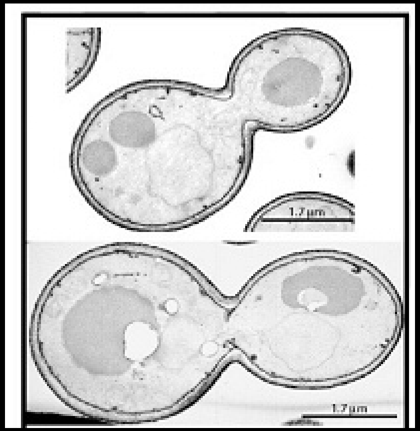
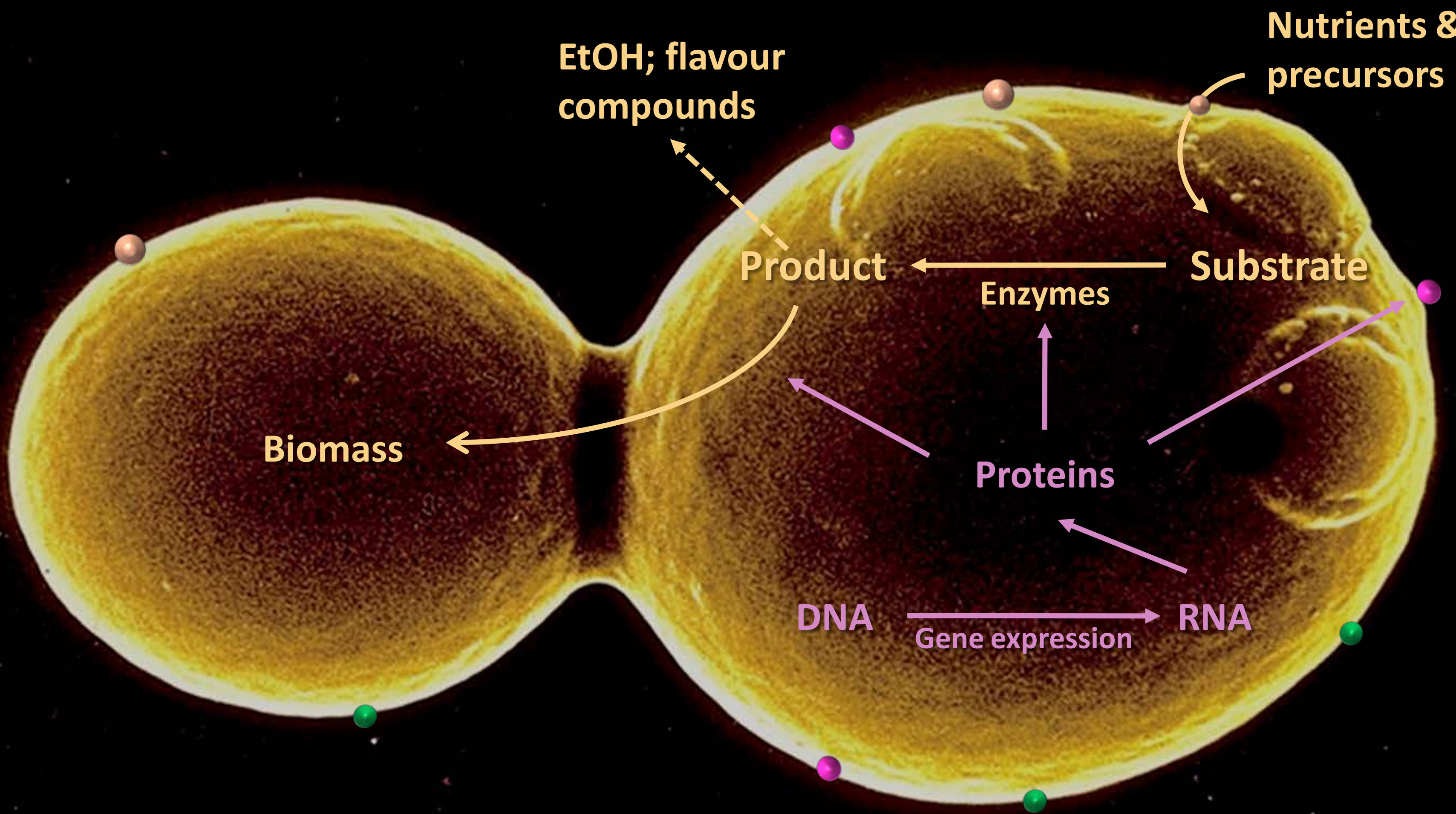
Wine yeast: *Saccharomyces*





What do yeasts do?



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Growth of *Saccharomyces cerevisiae*

Parameter	Optimal growth
Temperature	25-30°C
pH	5.0 – 5.5
Ethanol concentration	< 1.4 % v/v
Nitrogen	Ammonia/Glutamine
Oxygen	Aerobic
Water activity	0.998

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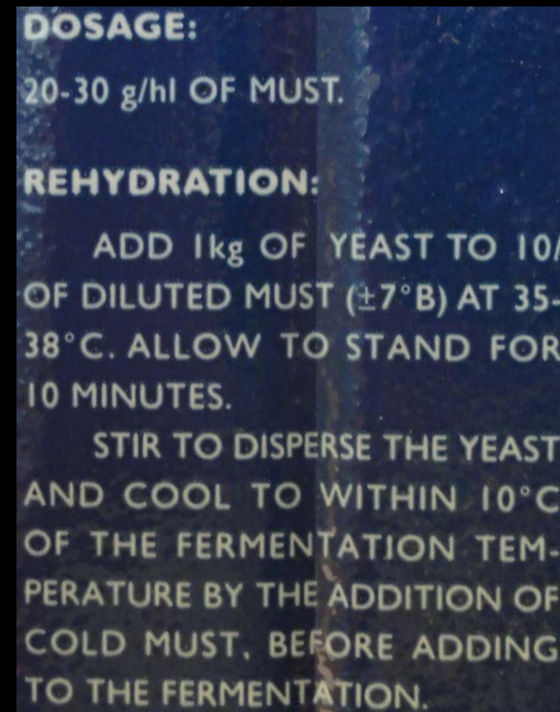
Impact of vinification on yeast growth

Parameter	Optimal growth	Vinification
Temperature	25-30°C	Variable
pH	5.0	< 3.4
Ethanol concentration	< 1.4 % v/v	Increasing to 11-16 % v/v
Nitrogen	Ammonia/Glutamine	Nitrogen depletion
Oxygen	Aerobic	Anaerobic
Water activity	0.998	Low (0.982-0.939)

ADY & inoculation



Typical wine fermentation

Increasing EtOH concentration

High solute concentration
Low pH
Low temperature
Oxygen

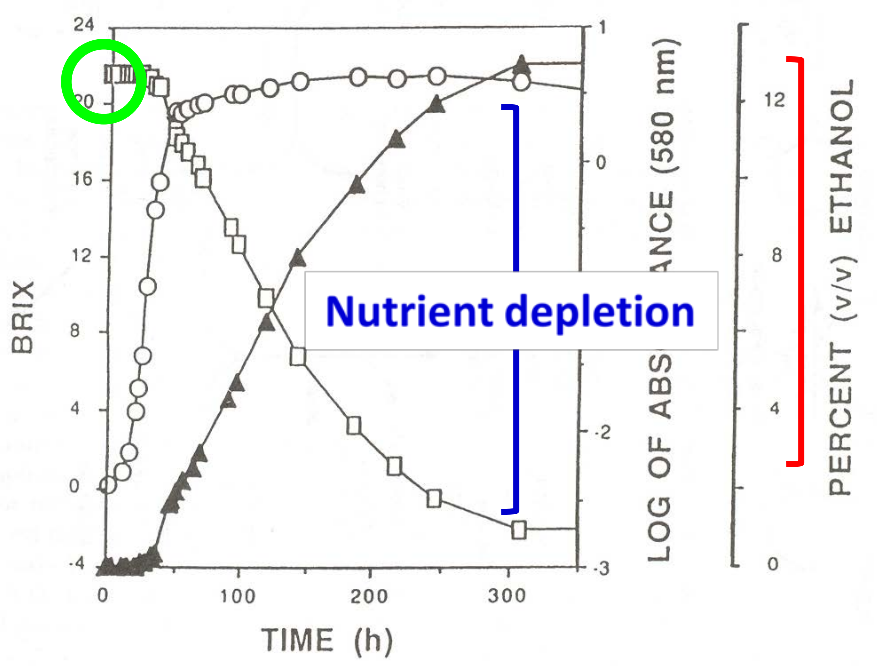


Fig. 4-11. Fermentation profile of grape juice. Absorbance (0); ethanol (▲); Brix (□).

Log of Absorbance = yeast cell density

In Boulton *et al*. (1998)

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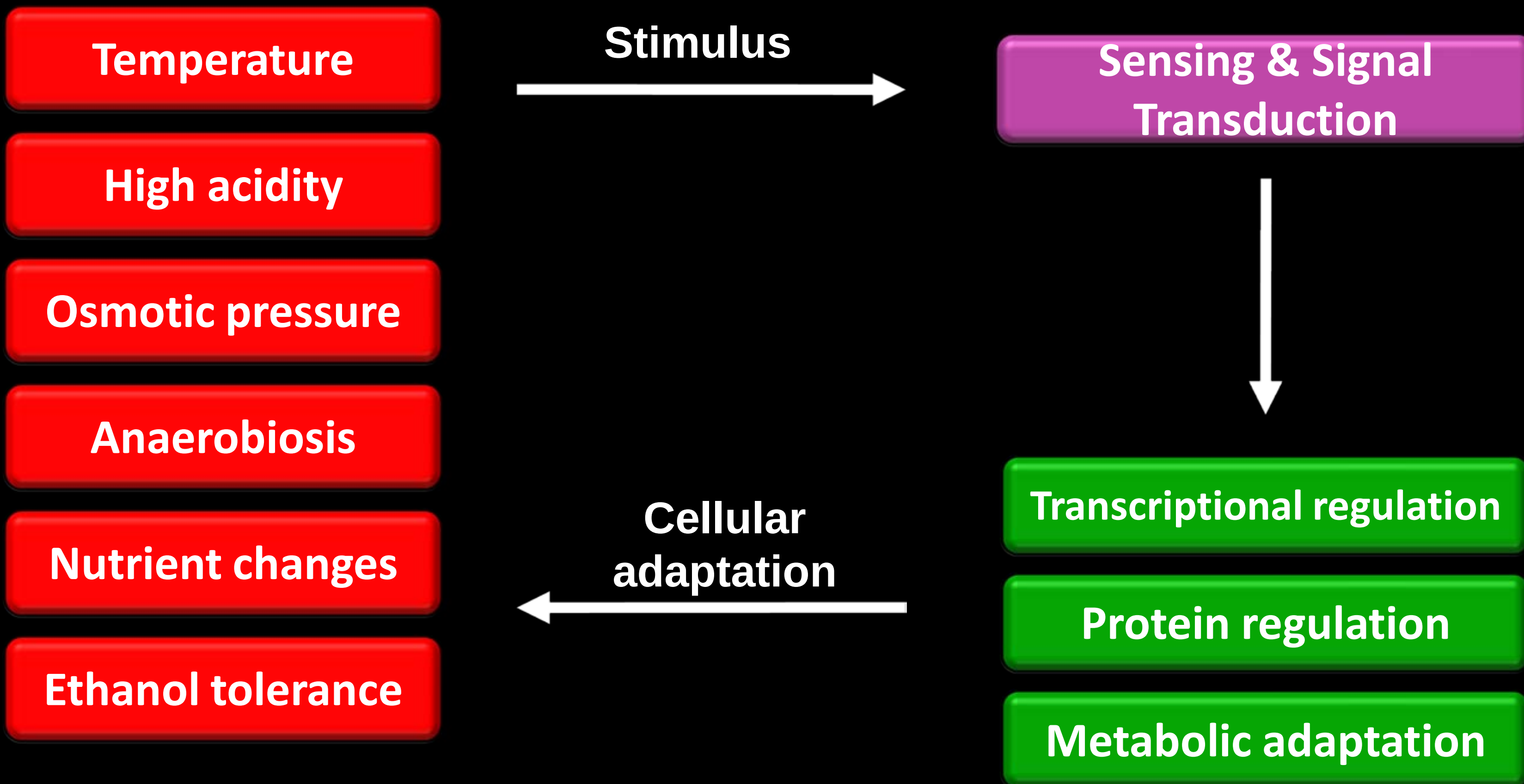
Fermentation stresses inhibit yeast performance

- Temperature
- High acidity
- Osmotic pressure
- Anaerobiosis
- Nutrient changes
- Ethanol tolerance

Growth, protection & survival

Physiological & metabolic adaptation

Adaptation of *S. cerevisiae* to its environment

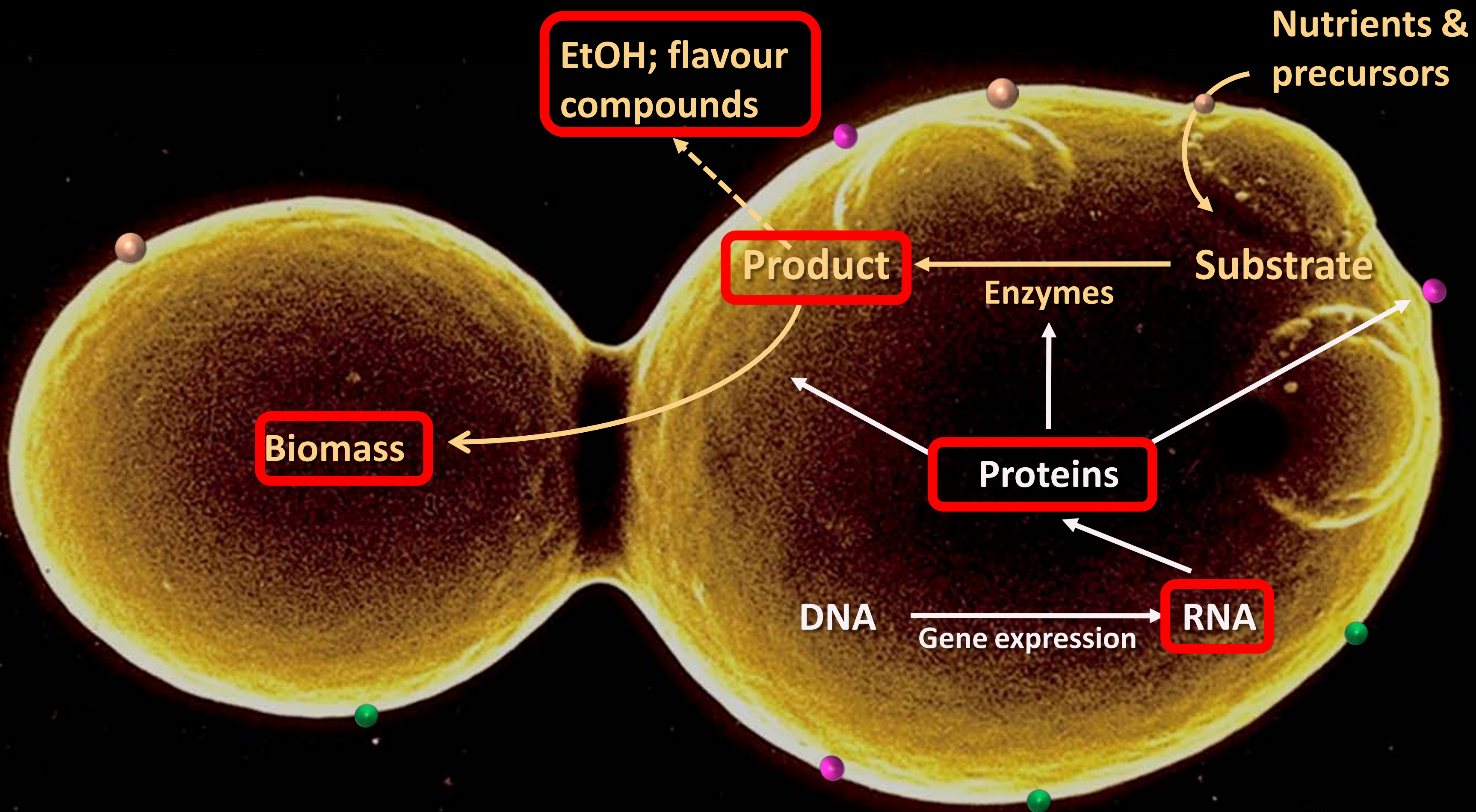


Impact of fermentation stresses

- Slow start
  - Increased lag phase
  - Wine, Icewine & Sparkling wine production
- Inefficient fermentations (stuck/sluggish)
  - Delay in sugar utilization and nutrient uptake; affects product quality
    - Off-flavour production
    - Spoilage organisms
- Winery efficiency
  - Cellar operations suffer; decreased/delayed production

  - Impacts bottom line……$$$

Measuring the yeast's response?



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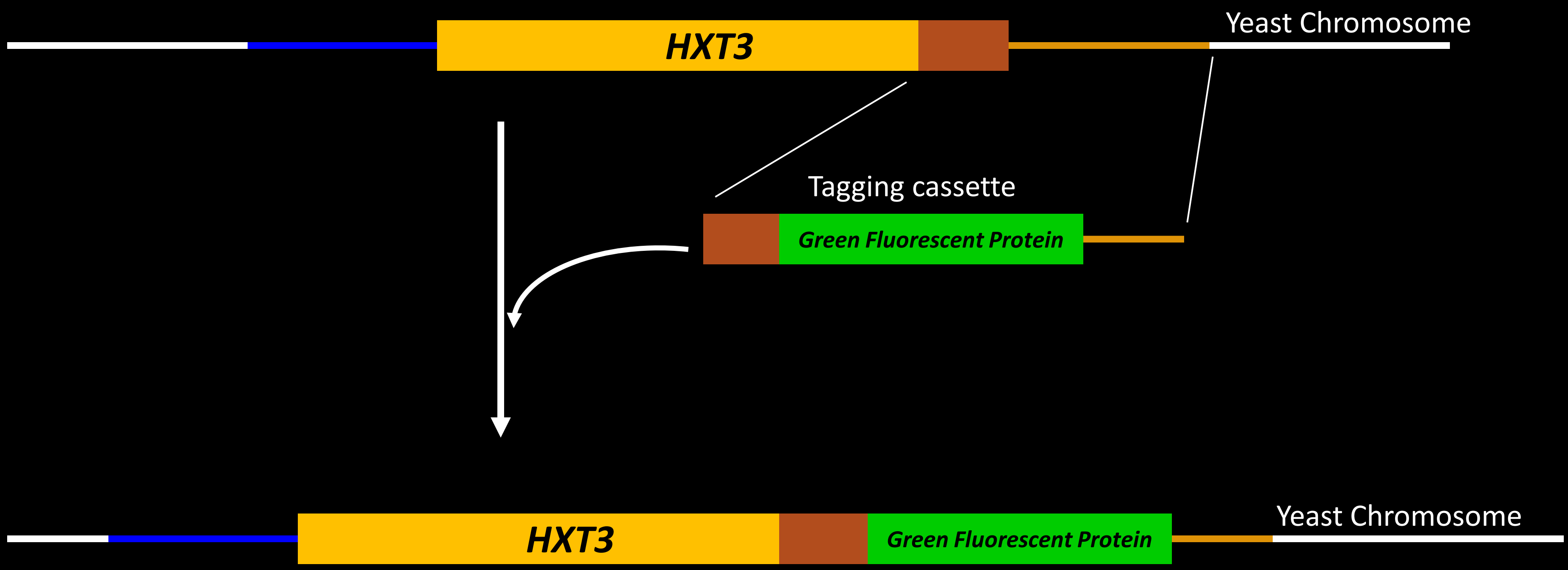
How can we "see" a protein in a yeast cell?



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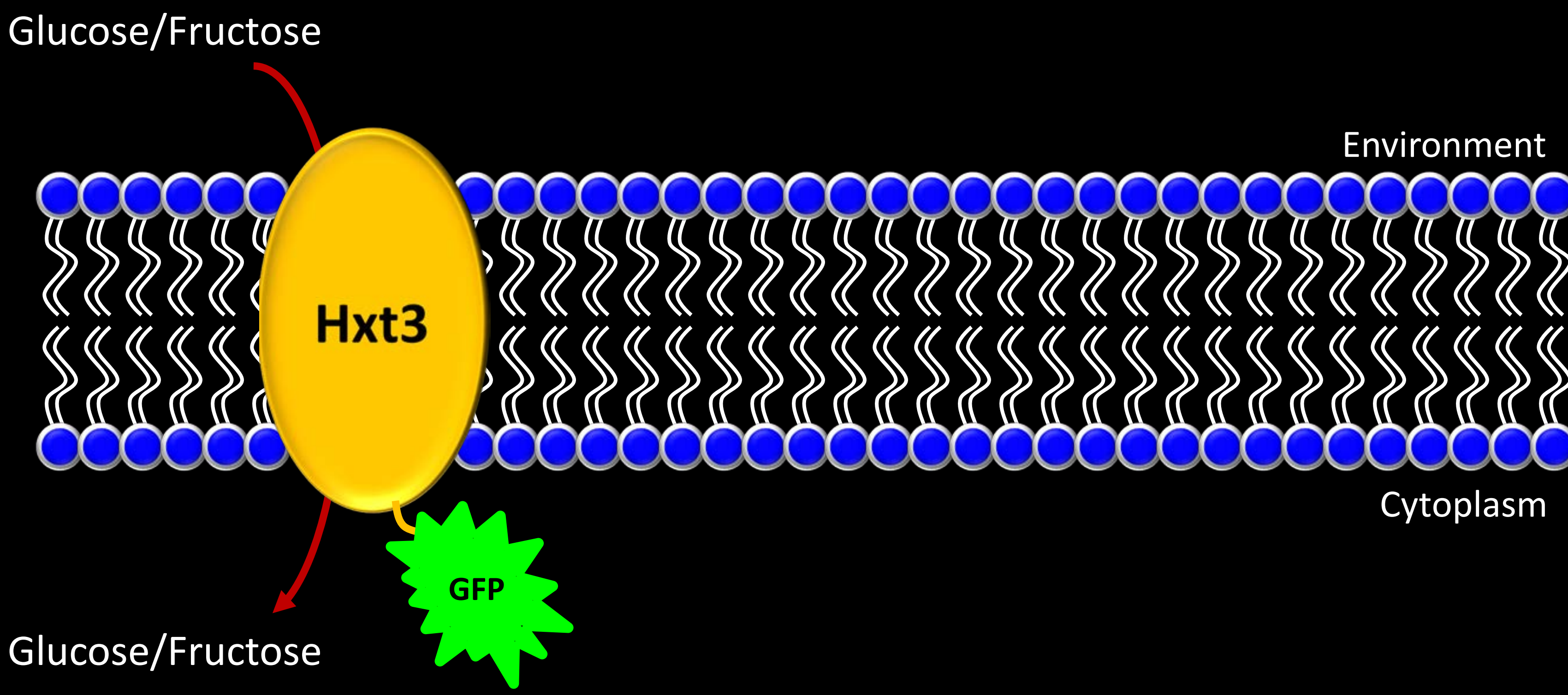
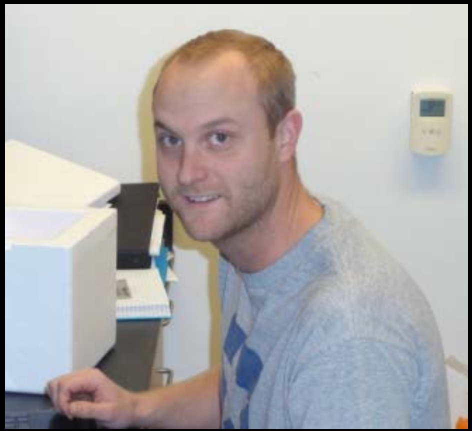
GFP-tagged hexose transporter



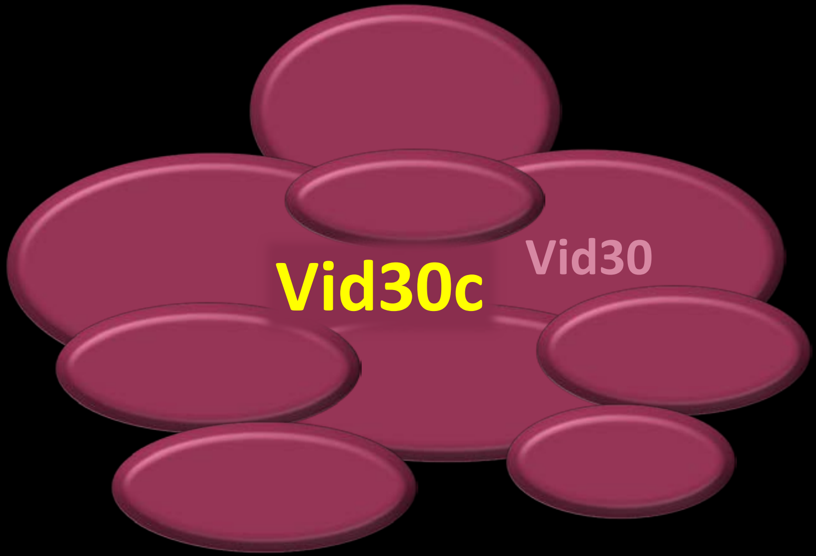
Vid30c & changing nutrient conditions

<u>*VID/GID* genes</u>

- Vid30 complex (Vid30c)
- Participate in adaptation to changing nutrient conditions
  - Involved in turnover of Hxt3 and Hxt7



Chris Snowdon



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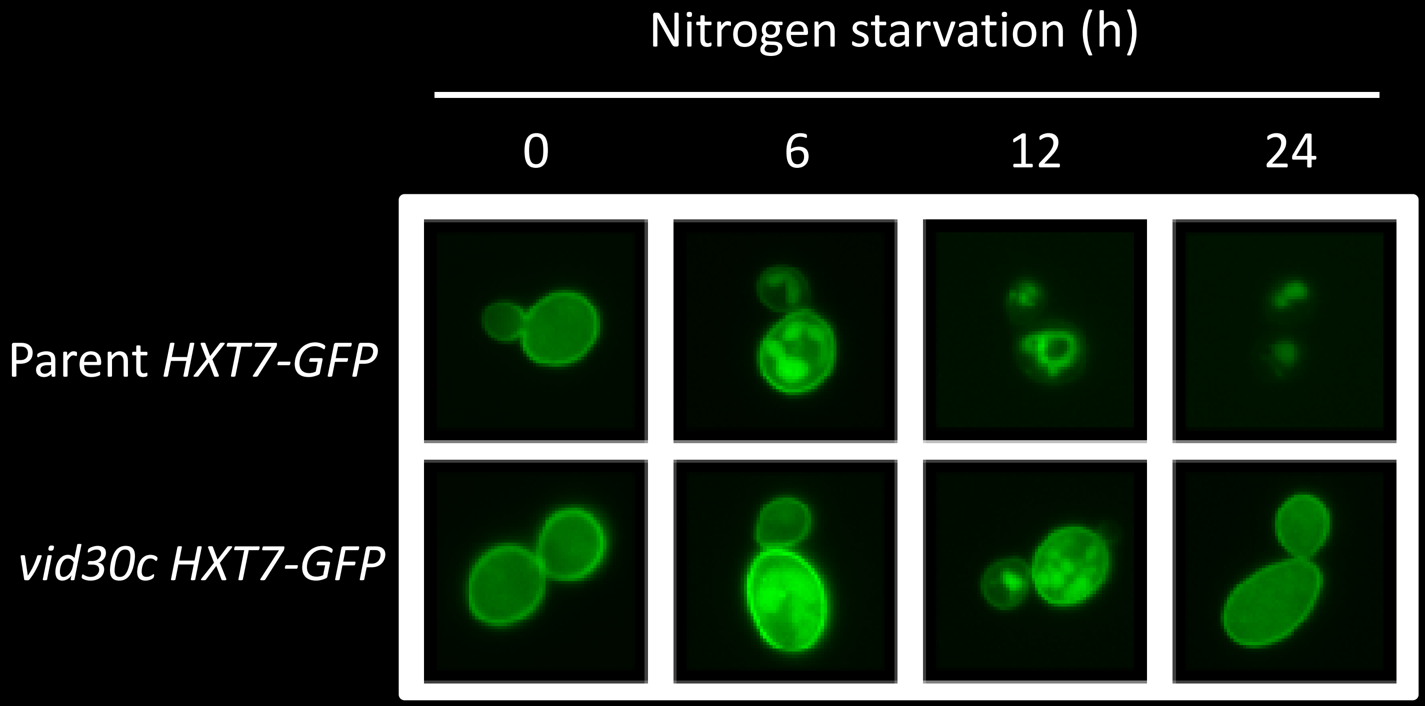
Vid30c impacts Hxt7 turnover



Snowdon *et al*. (2008) *FEMS Yeast Res* 8:204-216

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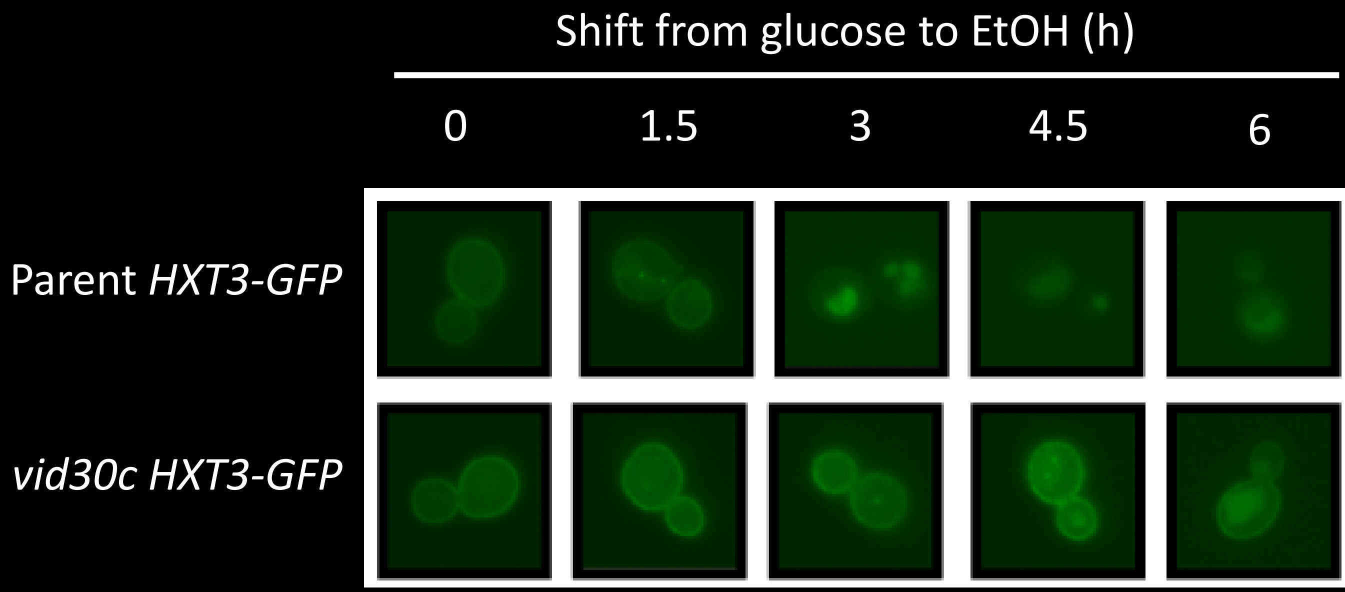
Vid30c participates in Hxt3 turnover



Snowdon and van der Merwe (2012) *PLoS ONE* 7(12): e50458

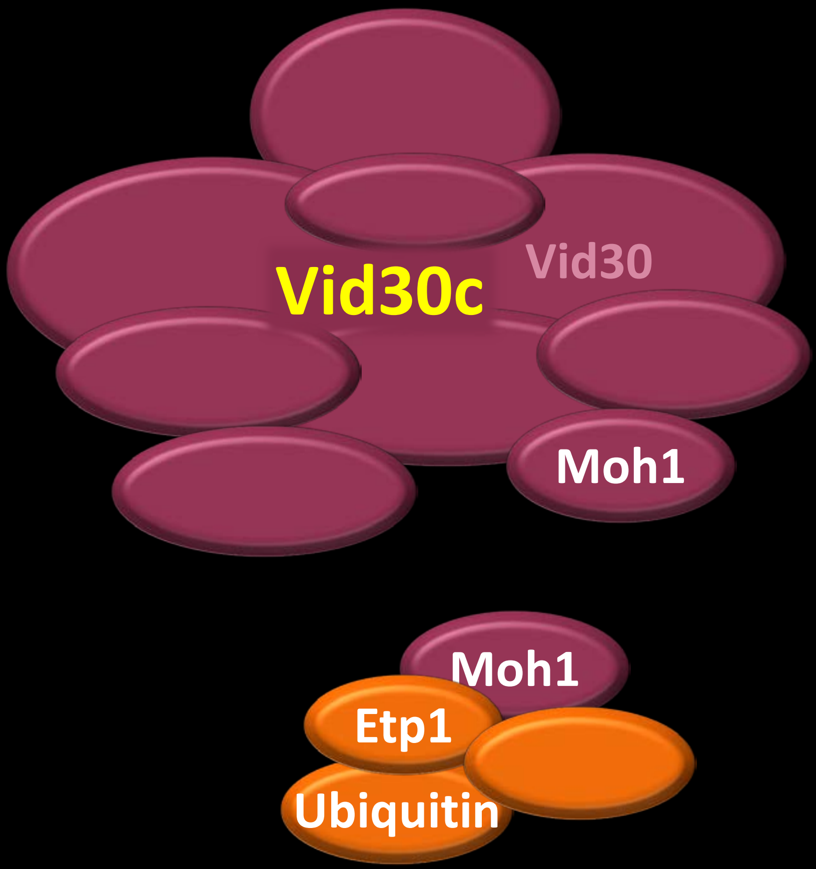
Vid30c and associated genes

VID/GID genes

- Vid30 complex (Vid30c)
- Participate in adaptation to changing nutrient conditions
  - Involved in turnover of Hxt3 and Hxt7

Etp1 complex

- Etp1 needed for ethanol tolerance



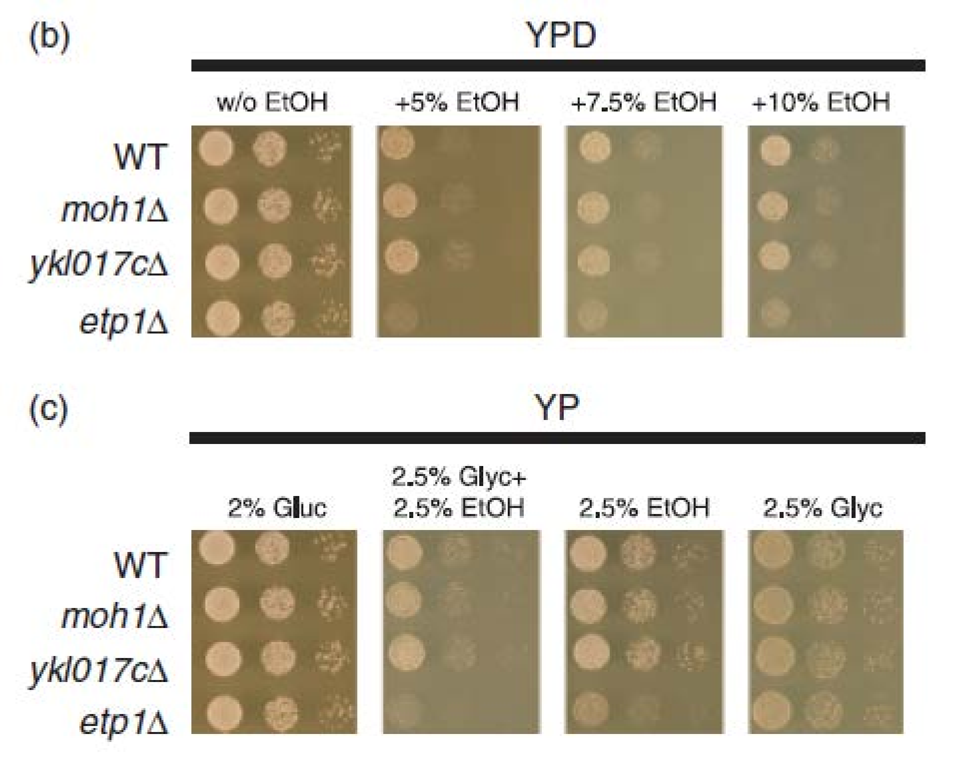
Ethanol tolerance

- 7.5% (w/v) ethanol considered ethanol stress
  - Impacts membrane fluidity
  - Denatures proteins
  - Greatly decreases cell viability
- Yeast's response
  - Adjusts membrane fluidity
  - Increase expression of chaperone proteins
    - Induces transcription of *HSP* genes

ETP1/YHL010c is a novel gene needed for the adaptation of *Saccharomyces cerevisiae* to ethanol

Christopher Snowdon, Ryan Schierholtz, Peter Poliszczuk, Stephanie Hughes & George van der Merwe

Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada



FEMS Yeast Res **9** (2009) 372–380

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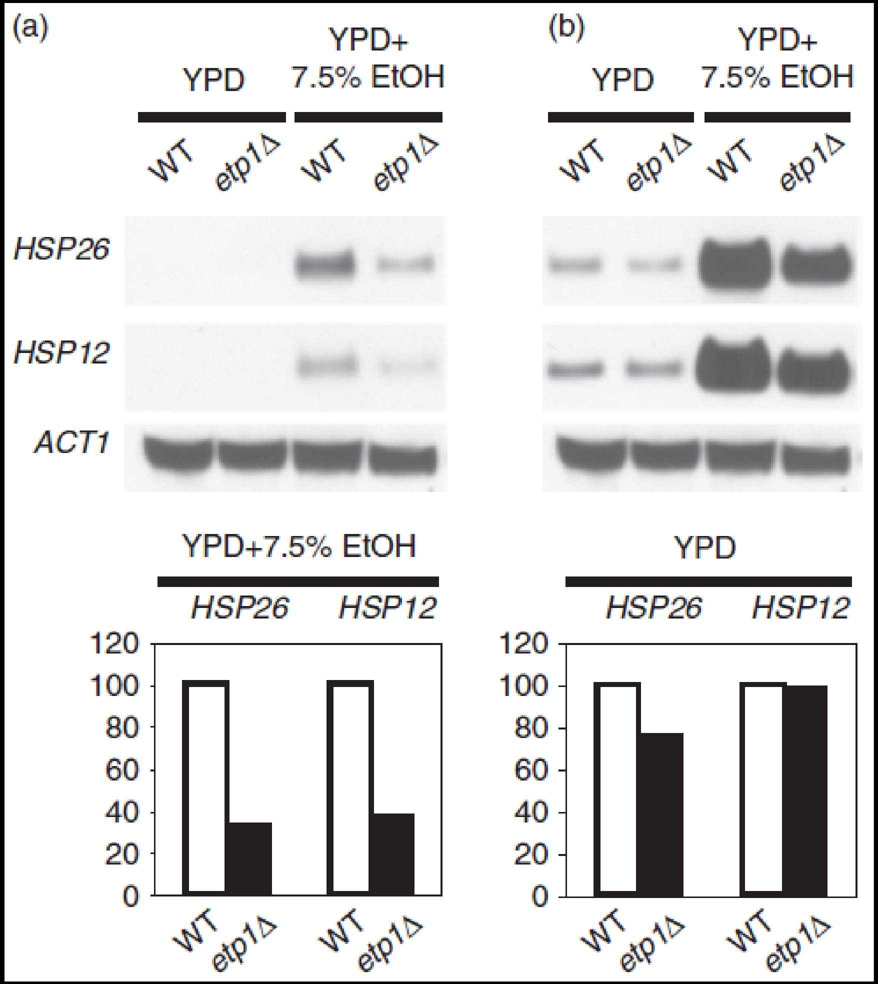
Etp1 is needed for *HSP* gene activation



Snowdon *et al*. (2009)

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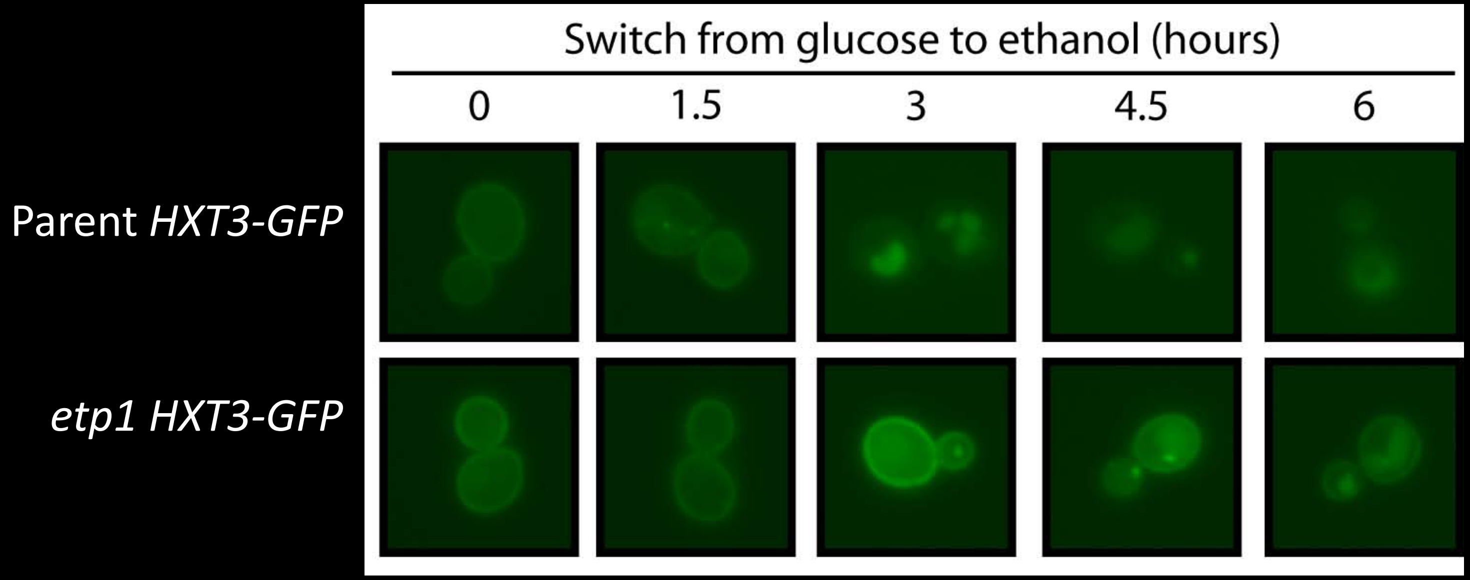
Etp1 is needed for Hxt3 turnover

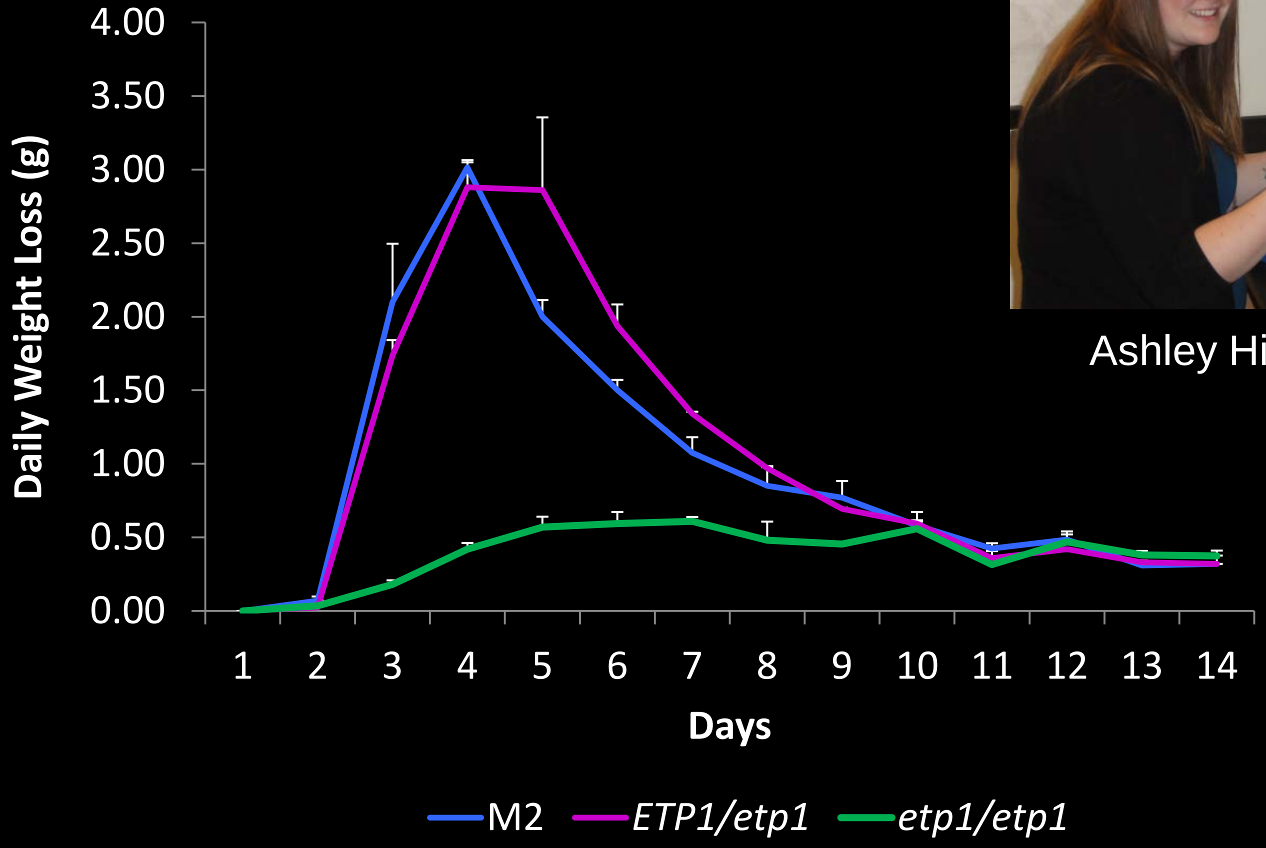


Snowdon *et al*. (2009)

Etp1 function in fermentation....

- Construct homo- and heterozygous mutants of *ETP1* in M2
- Chardonnay fermentations
- Hypothesis:
  - Needed for ethanol tolerance
  - Expects homozygous mutant to ferment well until high levels of ethanol is produced before mutant stops fermenting
    - Function when ethanol levels are high (around 7.5%)
    - Impact *HSP* gene expression later in fermentation

Etp1 is essential for efficient Chardonnay fermentation



Ashley Hillier

Hillier and GvdM (unpublished)

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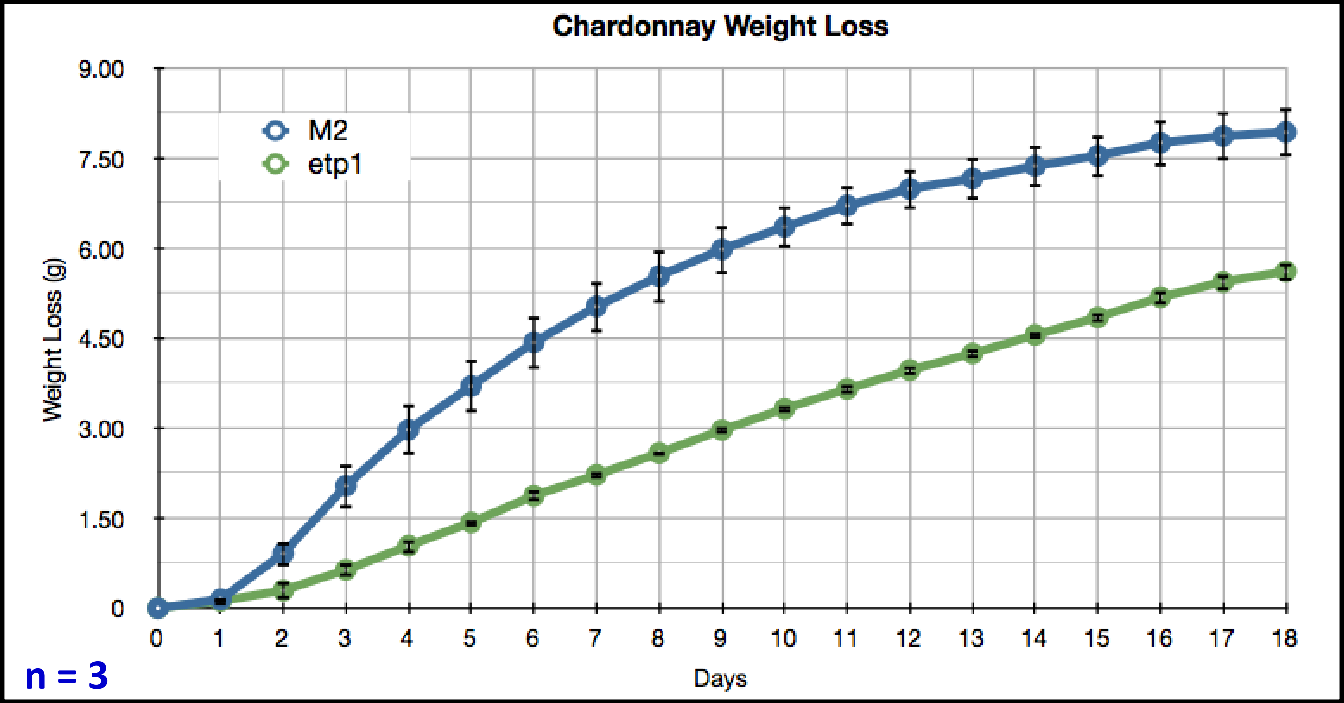
ETP1 needed for early adaptation to Chardonnay fermentation



Hillier and GvdM (in preparation)

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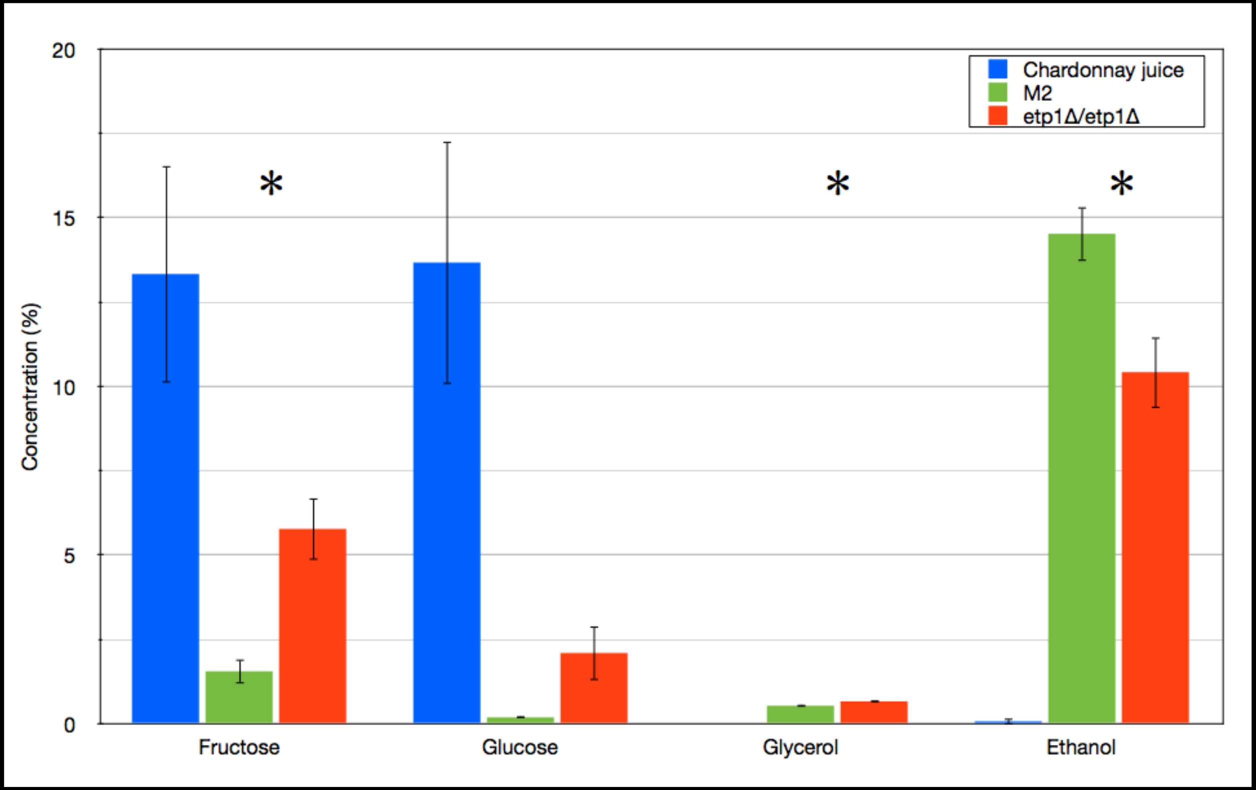
Loss of *ETP1* perturbs sugar metabolism during fermentation



* p-value ≤ 0.05

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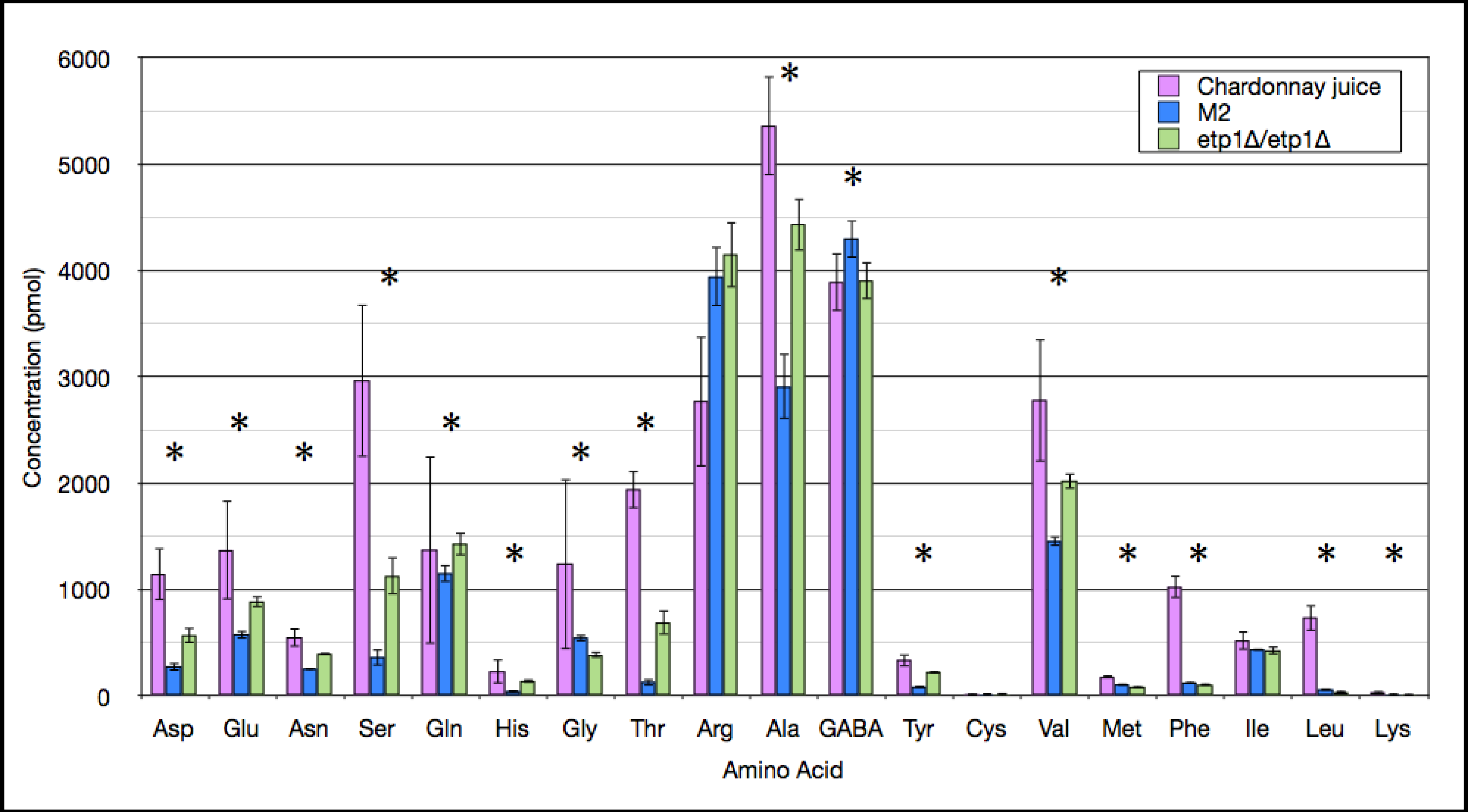
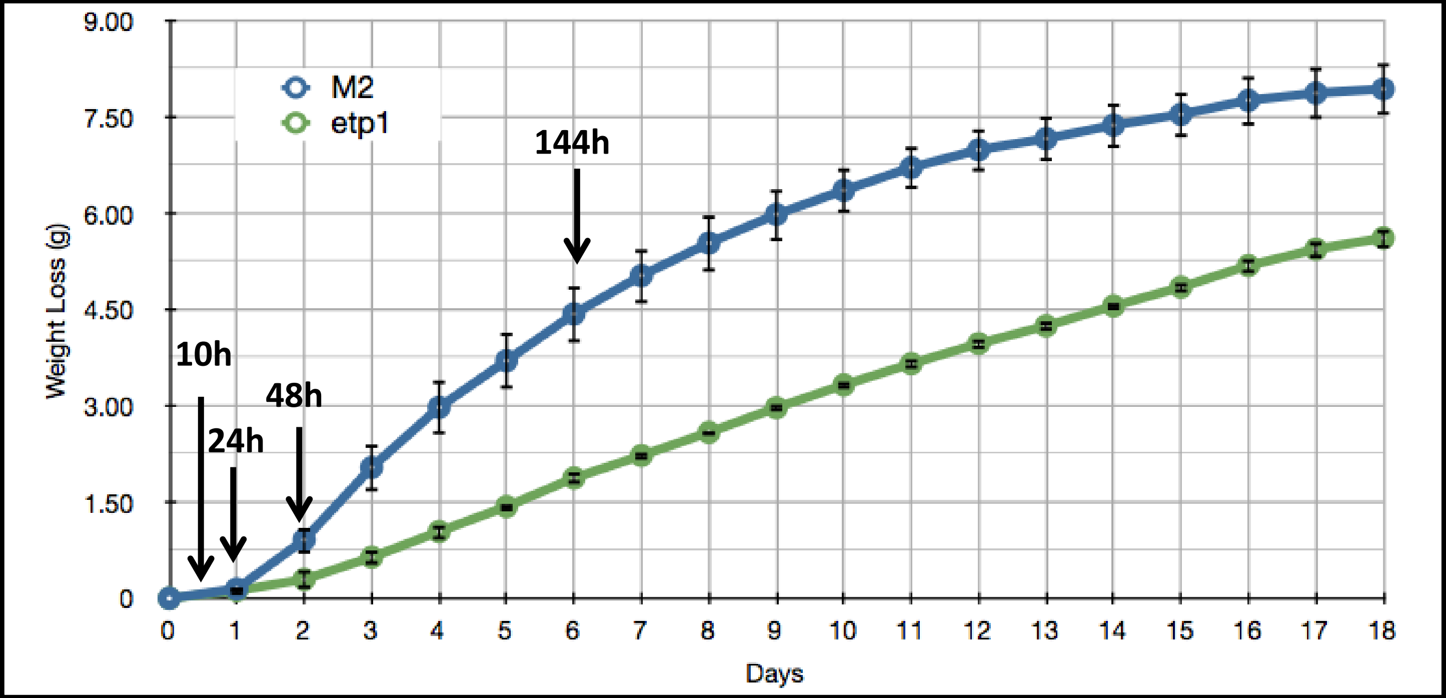
Loss of *ETP1* perturbs amino acid metabolism during fermentation



* p-value ≤ 0.05

Hillier and GvdM (in preparation)

ETP1 & transcriptional adaptation to Chardonnay fermentation



Biological triplicates; n = 3

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ETP1 impacts gene expression early in Chardonnay fermentation

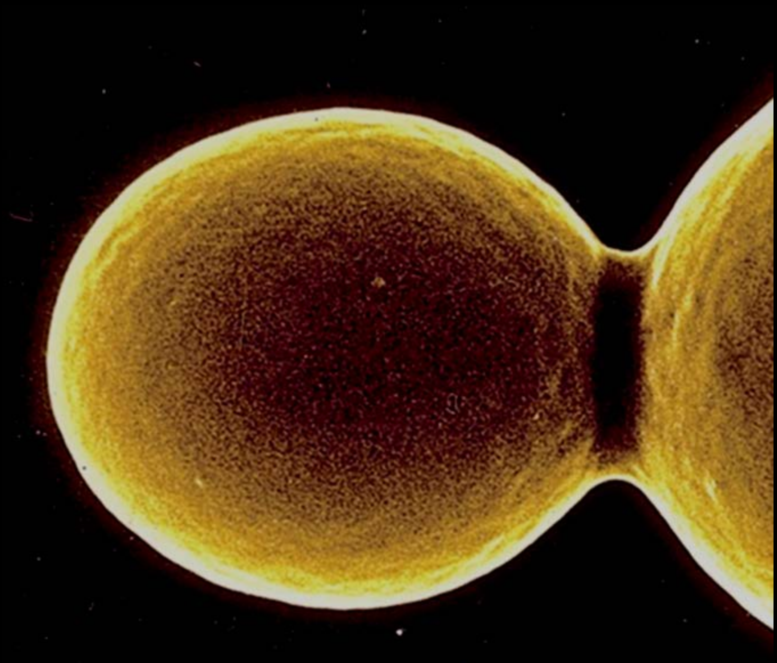
# genes compared to parent:	10 hours	24 hours	48 hours	144 hours
Higher in *etp1/etp1*	101	376	493	870
Lower in *etp1/etp1*	142	227	374	635
Total genes	243	603	867	1505

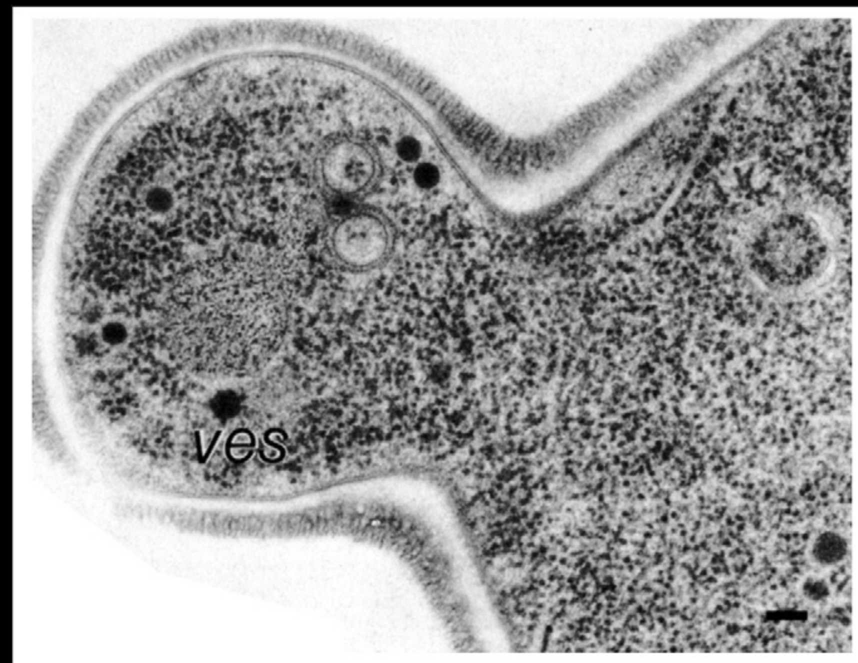
All genes: p-value ≤ 0.05; fold-change > 2

Groups of related genes affected by *ETP1* deletion

# genes in *etp1/etp1* compared to parent:	**10 hours**		**24 hours**		**48 hours**		**144 hours**	
	Up	Down	Up	Down	Up	Down	Up	Down
Amino acid & Nitrogen metabolism	2	13	17	15	35	19	58	31
Cold & anaerobiosis	0	5	3	17	1	19	2	11
Cell wall	6	1	20	1	30	2	15	8

Yeast cell wall





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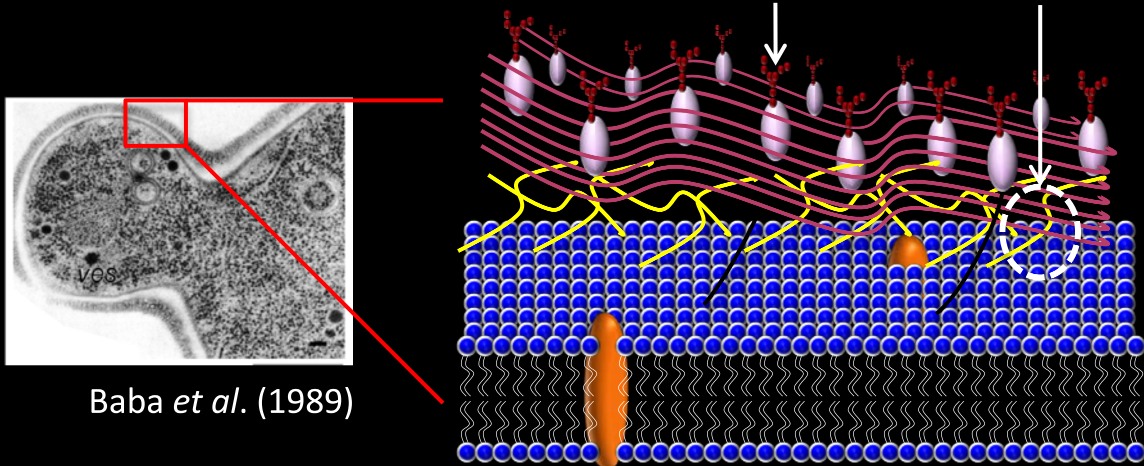
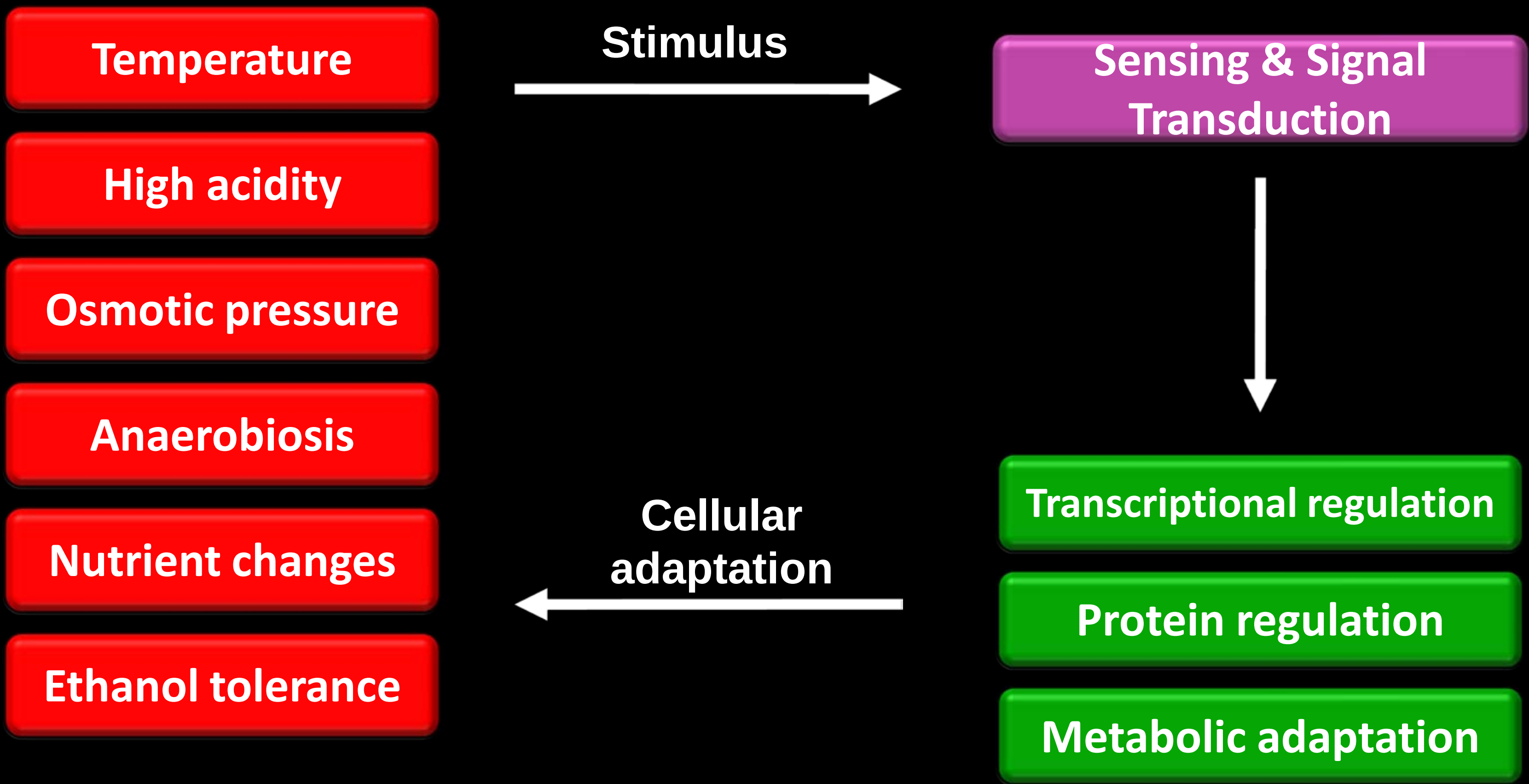
Yeast cell wall



Baba *et al*. (1989)

Adaptation of *S. cerevisiae* to its environment



Physicochemical factors and wine production: Cold & Anaerobiosis

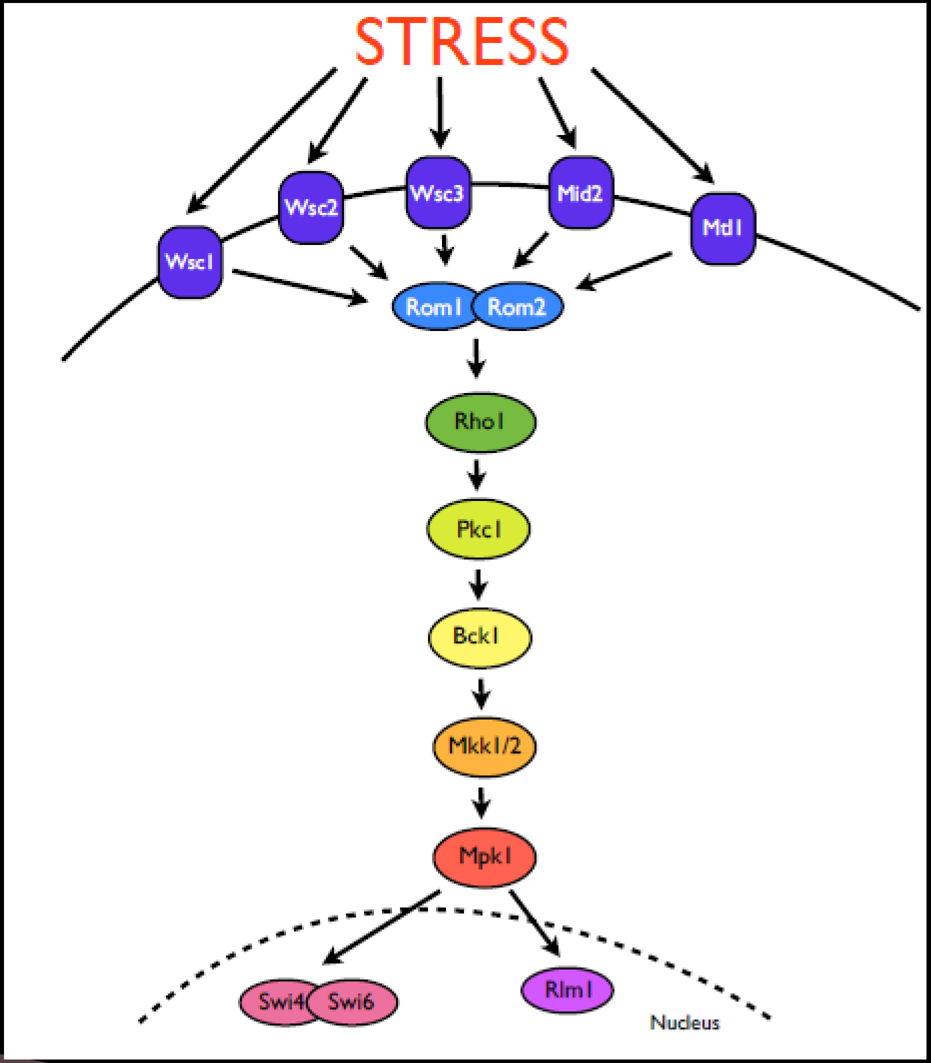
- Temperature of grape must during fall harvest
  - Cold soak
  - Colder fermentations for white wines
- Oxygen as major threat to wine production
  - Oxidation of flavour compounds
  - Low levels of dissolved oxygen at start of fermentation quickly scavenged by yeast following inoculation
  - "Oxygenation" during wine production
    - Pump-overs; micro-oxygenation; yeast RAPIDLY consumes oxygen during fermentation

Adaptation of yeast to cold and anaerobic conditions

- Impact on plasma membrane
  - Decrease in fluidity; decreased membrane function
  - Inability to produce new membrane lipids in absence of oxygen
  - Alteration to existing lipid composition to increase fluidity and membrane function

- Remodeling of cell wall
  - Alteration of cell wall components and proteins
  - Induced transcription of cell wall mannoprotein genes
    - *DAN/TIR/PAU*

Sensing cell wall stress

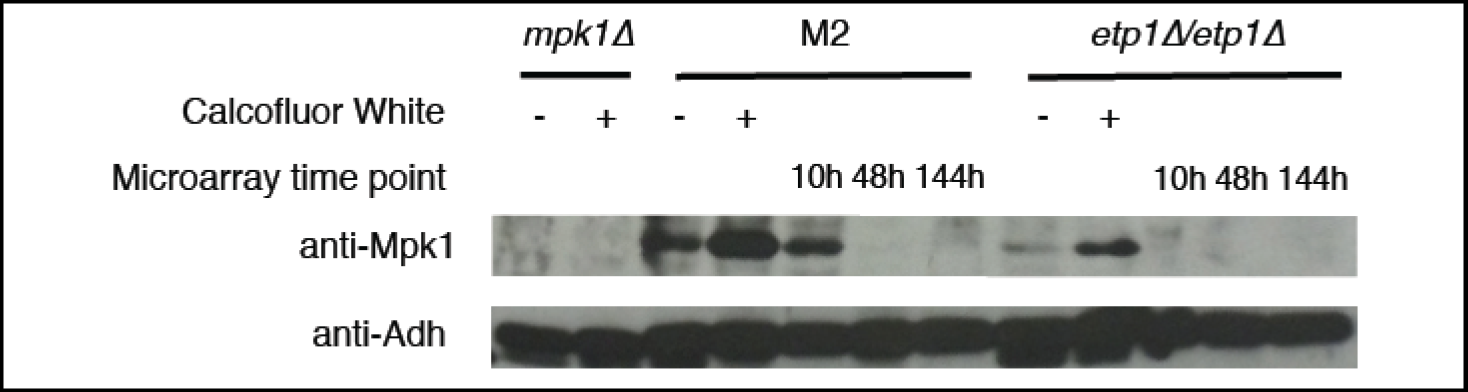


Etp1 impacts gene expression during fermentation

Genes	Time points				Gene Description
	10h	24h	48h	144h	
WSC2	.	.	+4.40	.	Sensor transducer proteins that sense cell wall stress and initiate the Protein Kinase C cascade (CWI pathway).
WSC3	.	.	.	+2.04	
MID2	.	.	+2.15	.	
MKK1	.	.	+2.09	.	Mitogen-activated kinase kinase (MEKK) involved in the cell wall integrity pathway.
SLT2/MPK1	.	.	+2.73	.	Terminal kinase in the cell wall integrity pathway.
CHS2	.	.	+4.92	+8.69	Encode for proteins involved in the synthesis, transport and deposition of chitin in the cell wall.
CHS7	.	.	+3.51	.	
CRH1	+3.29	+4.16	+5.71	.	
YEA4	.	.	+2.38	+2.35	
RCR1	.	+2.03	+2.28	+3.34	
KRE6	.	+2.40	.	.	Encode a group of proteins involved in beta-glucan assembly and transport to the cell wall.
KRE9	.	+2.14	+2.81	.	
KRE11	+2.02	+2.10	+3.10	.	
KEG1	.	.	+2.41	+2.51	
EXG1	.	+3.42	+3.77	.	
EXG2	.	+2.13	+4.96	+2.69	
GAS3	.	+3.09	+8.81	.	
GAS5	.	+3.86	+5.10	.	

Hillier and GvdM (in preparation)

Etp1 impacts levels of Mpk1



Genes	Time points				Gene Description
	10h	24h	48h	144h	
SLT2/MPK1	.	.	+2.73	.	Terminal kinase in the cell wall integrity pathway. Responsible for activating transcription factors and cell wall stress response genes.

Hillier and GvdM (in preparation)

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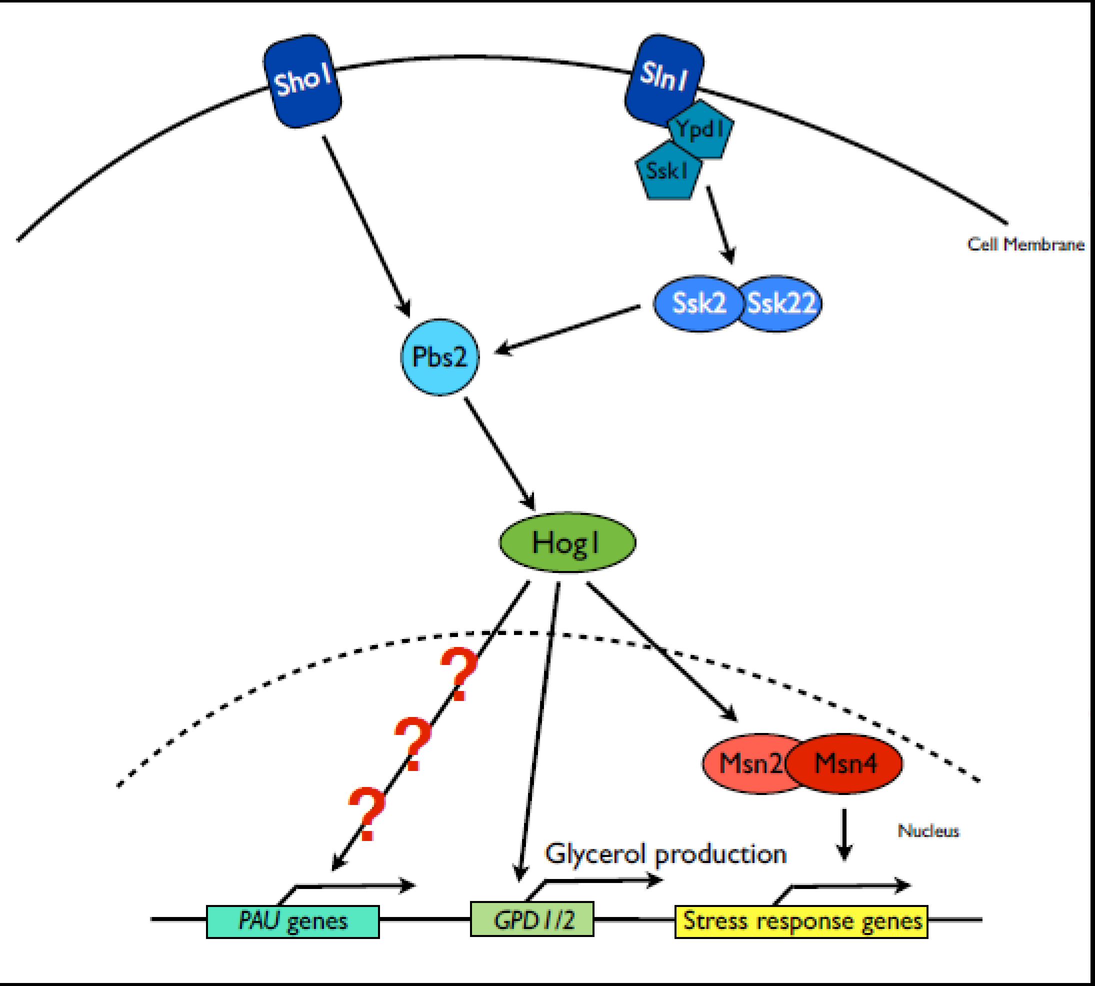
Sensing high sugars and anaerobic environments……



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FEMS Yeast Res **8** (2008) 374–385

Stress-induced production, processing and stability of a seripauperin protein, Pau5p, in *Saccharomyces cerevisiae*

Zongli Luo & Hennie J.J. van Vuuren

Wine Research Centre, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada

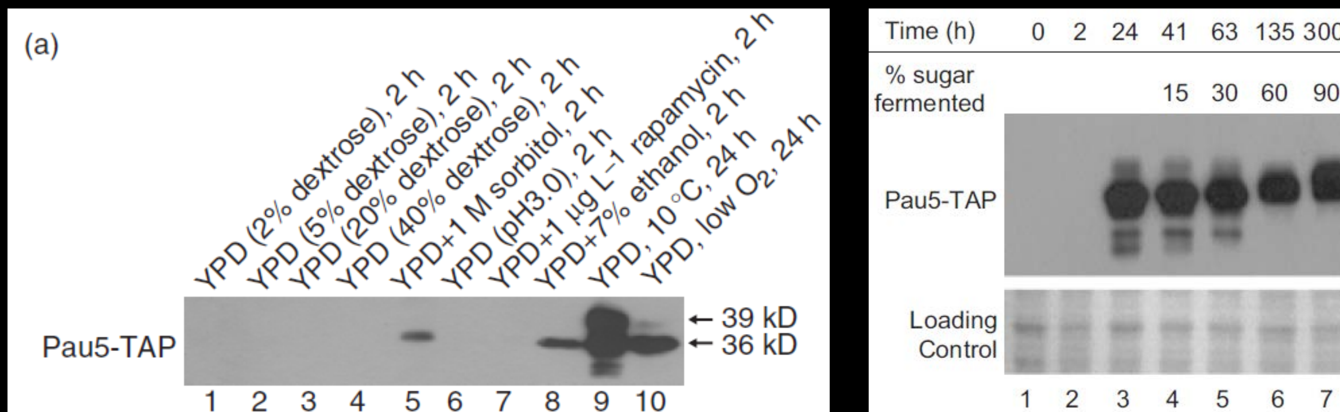


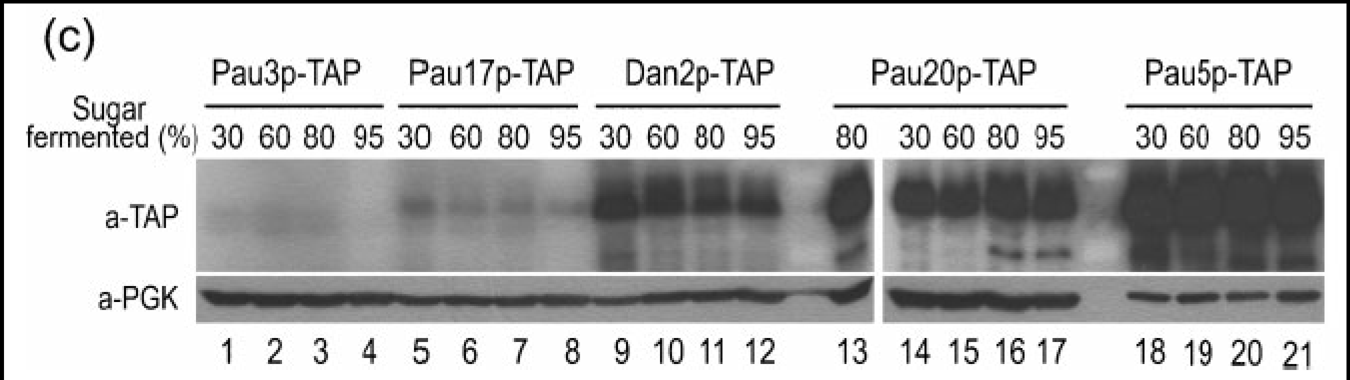
Fig. 3. Immunoblotting of cell lysates from wine yeast strain LY15 (chromosomally encoding Pau5-TAP). Cells were collected at various time points as indicated during Chardonnay must fermentations. (a) and (b) are results from two independent fermentations. Loading controls were visualized by staining as described in 'Materials and methods'.

Microbiology (2009), **155**, 4036–4049

Functional analyses of *PAU* genes in *Saccharomyces cerevisiae*

Zongli Luo and Hennie J. J. van Vuuren

Wine Research Centre, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

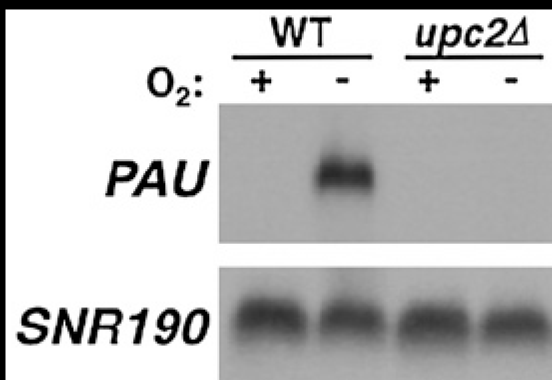


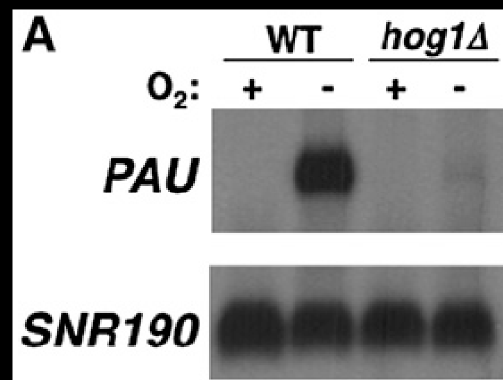
Genetics 188: 325–338 (June 2011)

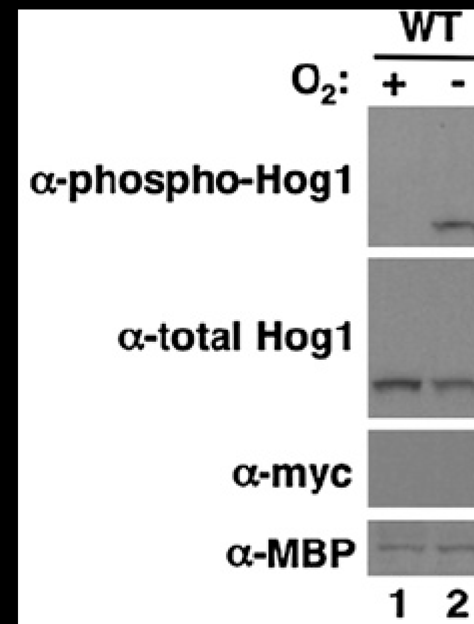
The Hog1 Mitogen-Activated Protein Kinase Mediates a Hypoxic Response in *Saccharomyces cerevisiae*

Mark J. Hickman,*,† Dan Spatt* and Fred Winston*,[1]

*Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115 and †Lewis–Sigler Institute and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544





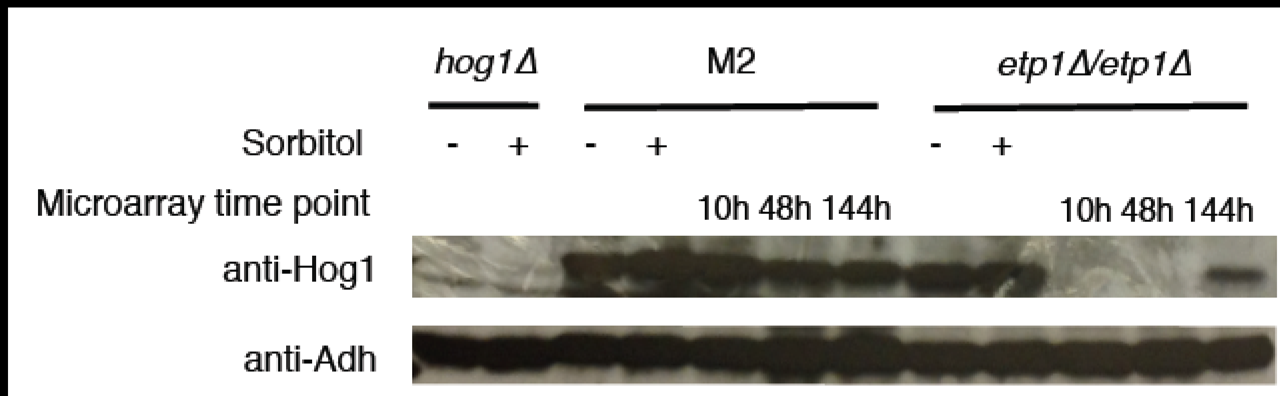


Etp1 impacts gene expression during fermentation

Genes	Time points				Gene Description
	10h	24h	48h	144h	
PAU1	.	-9.96	-11.01	.	A family of genes encoding seripauperin proteins, shown to be induced under anaerobic conditions as well as low temperatures and cold shock
PAU2	.	-12.72	-21.82	-3.43	
PAU5	.	-10.80	-16.84	-6.54	
PAU7	.	-15.96	-36.00	-8.14	
PAU8	.	-11.05	-11.62	.	
PAU10	.	-10.66	-13.25	.	
PAU15	.	-25.65	-25.09	-2.02	
PAU17	.	-14.89	-15.90	-7.51	
PAU18	.	-5.18	-4.84	.	
PAU20	.	-4.32	-3.64	.	
PAU21	-3.40	-2.50	-4.77	.	
PAU23	.	-10.54	.	-2.18	
PAU24	.	-26.63	-51.39	-3.47	
DAN1	-5.15	-9.72	-18.48	-19.90	Cell wall mannoproteins expressed during cold shock and anaerobiosis.
DAN4	.	.	.	-2.19	
TIR3	.	.	-2.33	.	
TIR4	-7.49	-2.34	-2.72	.	
UPC2	.	.	-4.53	-5.39	Regulator of the *DAN/TIR* genes.

Hillier and GvdM (in preparation)

Etp1 impacts levels of Hog1



Genes	Time points				Gene Description
	10h	24h	48h	144h	
PAU1	.	-9.96	-11.01	.	
PAU2	.	-12.72	-21.82	-3.43	
PAU5	.	-10.80	-16.84	-6.54	
PAU7	.	-15.96	-36.00	-8.14	
PAU8	.	-11.05	-11.62	.	
PAU10	.	-10.66	-13.25	.	A family of genes encoding seripauperin proteins, shown to be induced under anaerobic conditions as well as low temperatures and cold shock
PAU15	.	-25.65	-25.09	-2.02	
PAU17	.	-14.89	-15.90	-7.51	

Hillier and GvdM (in preparation)

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Conclusions

- *ETP1* is needed for a normal fermentation to occur
  - Significant impact on transcriptional adaptation process
- *ETP1* deletion affects protein levels of Hog1 and Mpk1 early in fermentation
  - Leads to significant down-regulation in *PAU* gene transcription early in fermentation
  - Cell remodelling genes are mis-regulated
- Etp1 is most likely involved in the ubiquitin-dependent turnover of proteins
  - Specific target(s)?

Acknowledgements

GvdM lab members

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Ashley Hillier
Stephanie Hughes
Erik Nielson
Stephanie Hallows
Peter Poliszczuk
Nate Ferguson

Collaborators

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Barry Shelp (U. of Guelph)
Hennie van Vuuren (UBC)
Terence van Rooyen (NCTW)
Hung Lee (U. of Guelph)

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CFI
NSERC
OMAFRA
Genome Canada
ORF-RE

Etp1 impacts gene expression sulphur metabolic genes

Genes	Time points				Gene Description
	10h	24h	48h	144h	
GAP1	.	+4.08	+4.22	+12.29	Amino acid permeases.
BAP2	.	+4.56	.	-2.83	
BAP3	.	.	.	-12.68	
DIP5	-3.65	.	+4.27	+5.48	
MET2	.	.	+2.08	-3.7	Involved in the biosynthesis and metabolism of methionine, as well as sulfate assimilation.
MET3	.	-2.68	+8.57	.	
MET4	.	.	.	-3.09	
MET5	.	.	+6.53	+2.28	
MET6	-2.33	-2.21	.	+2.33	
MET8	-4.02	.	.	.	
MET13	-6.64	-2.18	.	.	
MET14	.	-2.34	+3.13	-2.35	
MET16	.	.	+2.28	-2.88	
MET31	.	+2.20	.	-2.28	
MET32	.	.	+4.48	-2.08	
SAM4	.	.	+3.35	+4.13	
MHT1	.	.	+3.32	.	

Hillier and GvdM (in preparation)

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