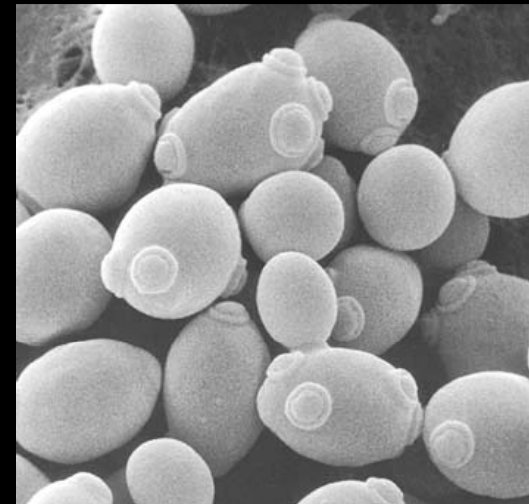
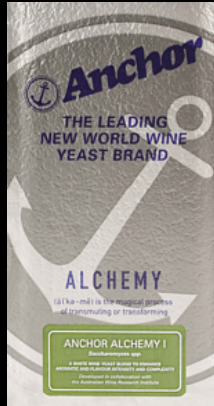


# Understanding the impact of fermentation stresses on yeast during vinification

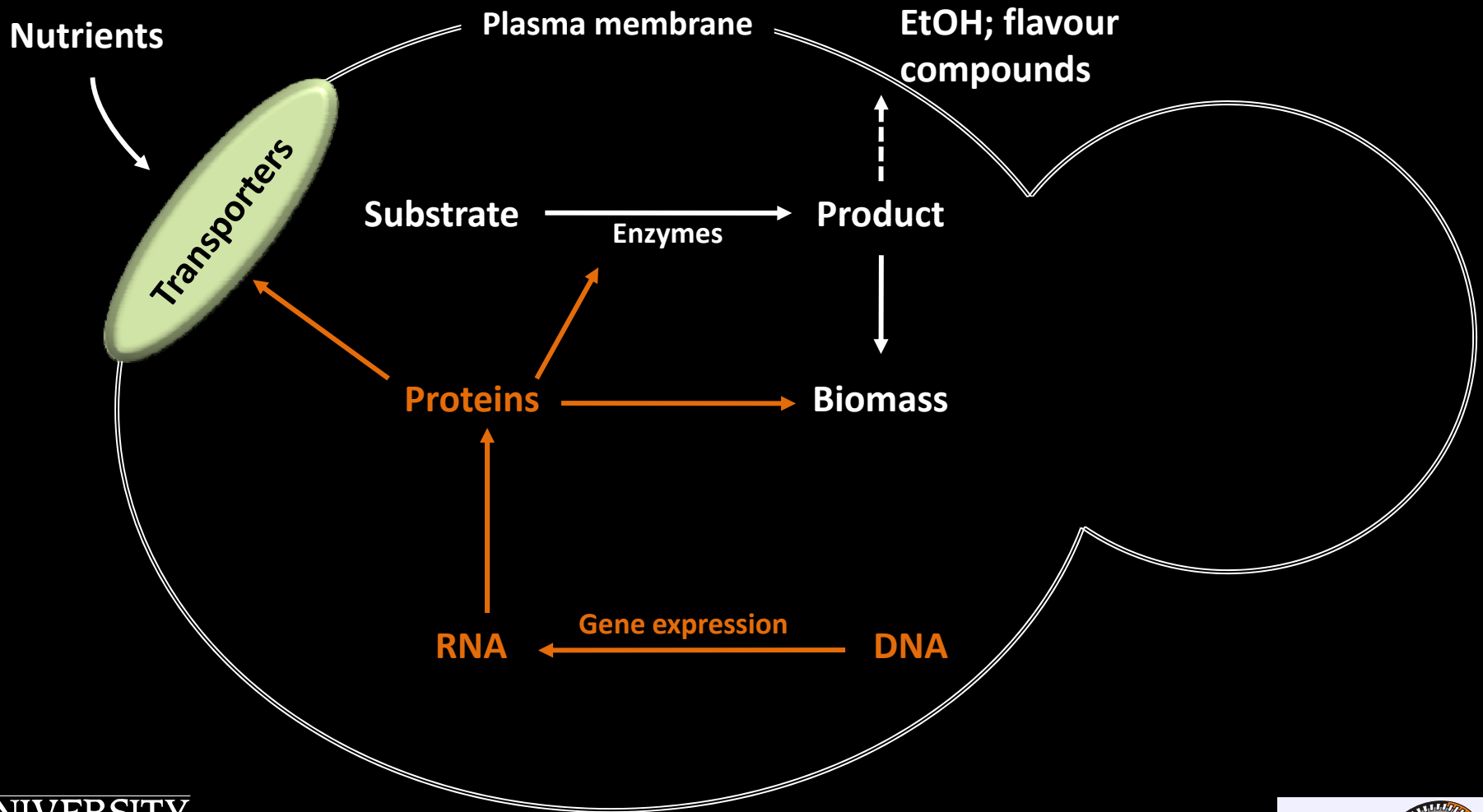
George van der Merwe  
Associate Professor

CCOVI - May 2010

# Wine yeast: *Saccharomyces*



# What do yeast do?



# Growth of *Saccharomyces cerevisiae*

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

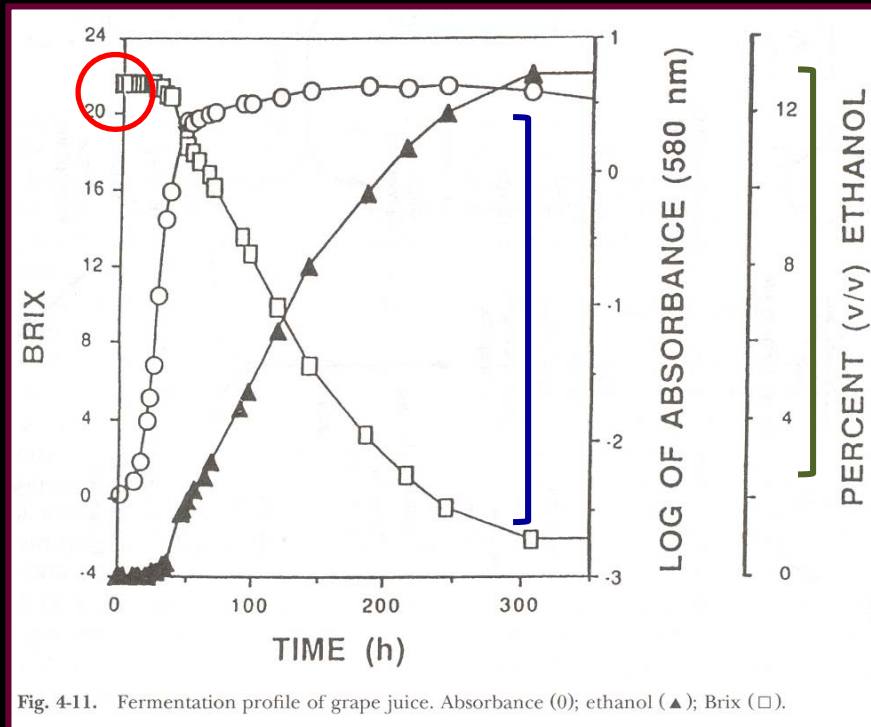
# Major constituents of grape juice

Compound	Percent
Water	76%
Glucose	8-13%
Fructose	8-13%
Tartaric acid	0.2-1.0%
Malic acid	0.1-0.8%
Citric acid	0.01-0.05%
Tannins (catechol, chlorogenic acid, caffeic acid)	0.01-0.1%
Nitrogenous compounds (amino acids and proteins)	variable
Other minerals (phosphates, sulphates)	Traces
B-group vitamins (thiamine, riboflavin, pyridoxine, nicotinic acid)	Traces
Ascorbic acid	Traces
Volatile aroma constituents	Traces
Colour constituents	Traces

# Typical wine fermentation

High solute concentration  
Low pH

Increasing EtOH concentration



Nutrient depletion

*In Boulton et al. (1998)*

Log of Absorbance = measures yeast cell density

# Impact of vinification on growth of *Saccharomyces cerevisiae*

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)

# Fermentation stresses inhibit yeast performance

Osmotic stress

Temperature extremes

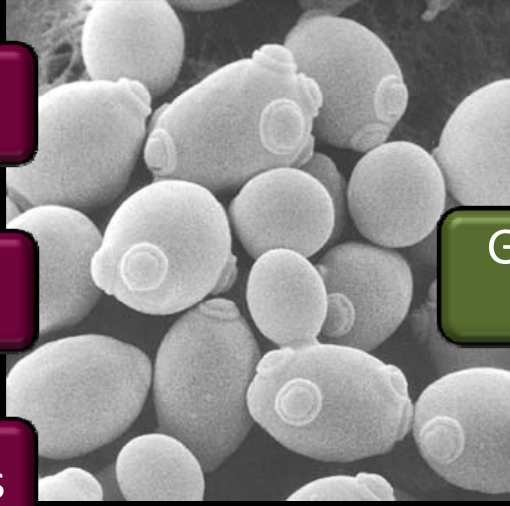
Weak acid stress

Ethanol toxicity

Nutrient deprivation

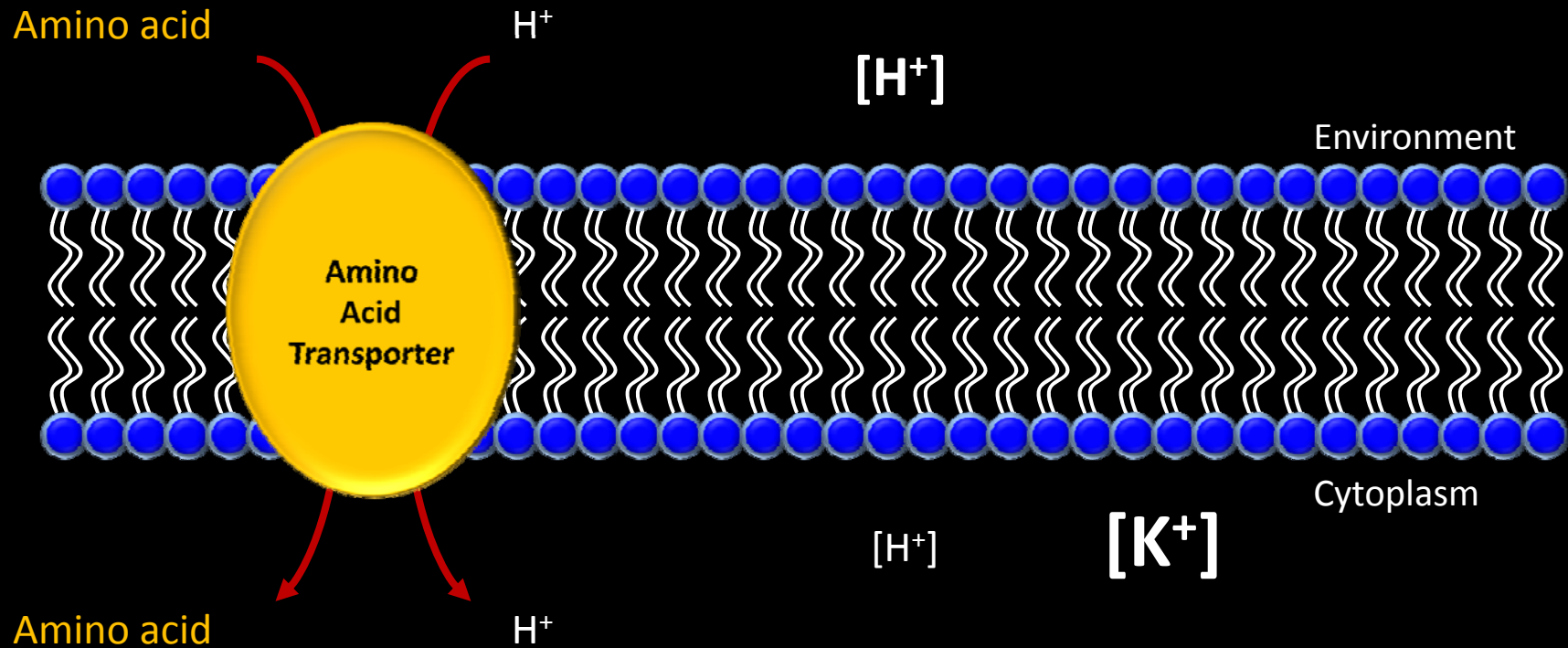
Growth, protection & survival

Physiological & metabolic adaptation

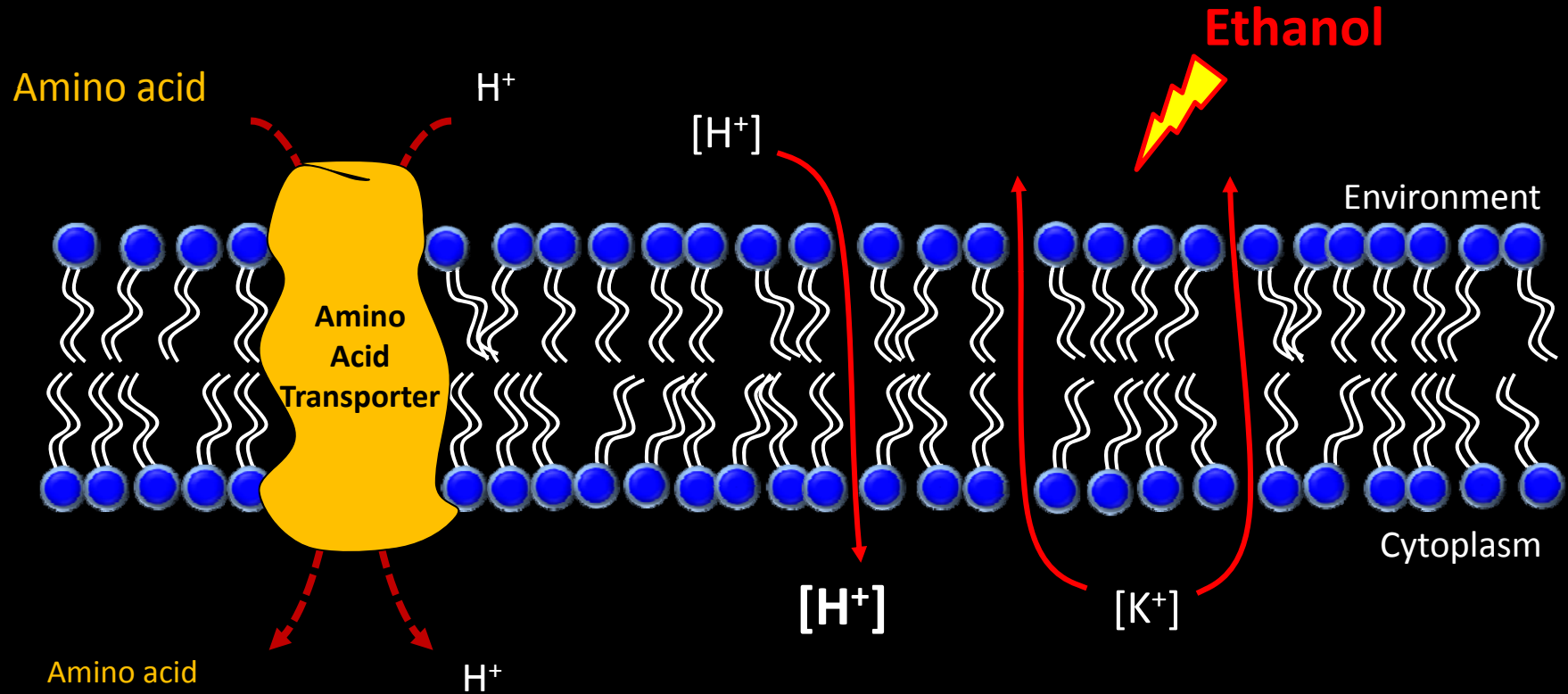




# Ion homeostasis and amino acid transport



# Fermentation stresses: Ethanol toxicity



# Response to ethanol toxicity

- Alteration in membrane composition
  - Lipid alteration and sterol (ergosterol) production
- Increased effort to restore intracellular pH
- Production of chaperones (HSPs) to assist in protein refolding
- Increased synthesis of trehalose
- Intracellular amino acid accumulation

# Fermentation stresses: Nutrient deprivation

- Nitrogen depletion
  - Turnover of hexose transporters needed for completion of fermentations; Stuck/sluggish fermentations
  - Production of sulphur-containing off flavours
  - Increased production of higher alcohols & isoacids
  - Decreased production of esters
- Vitamin depletion
  - Off-flavour production
- Lipid depletion
  - Inability to restructure membrane; Stuck/sluggish fermentation

# Impact of fermentation stresses

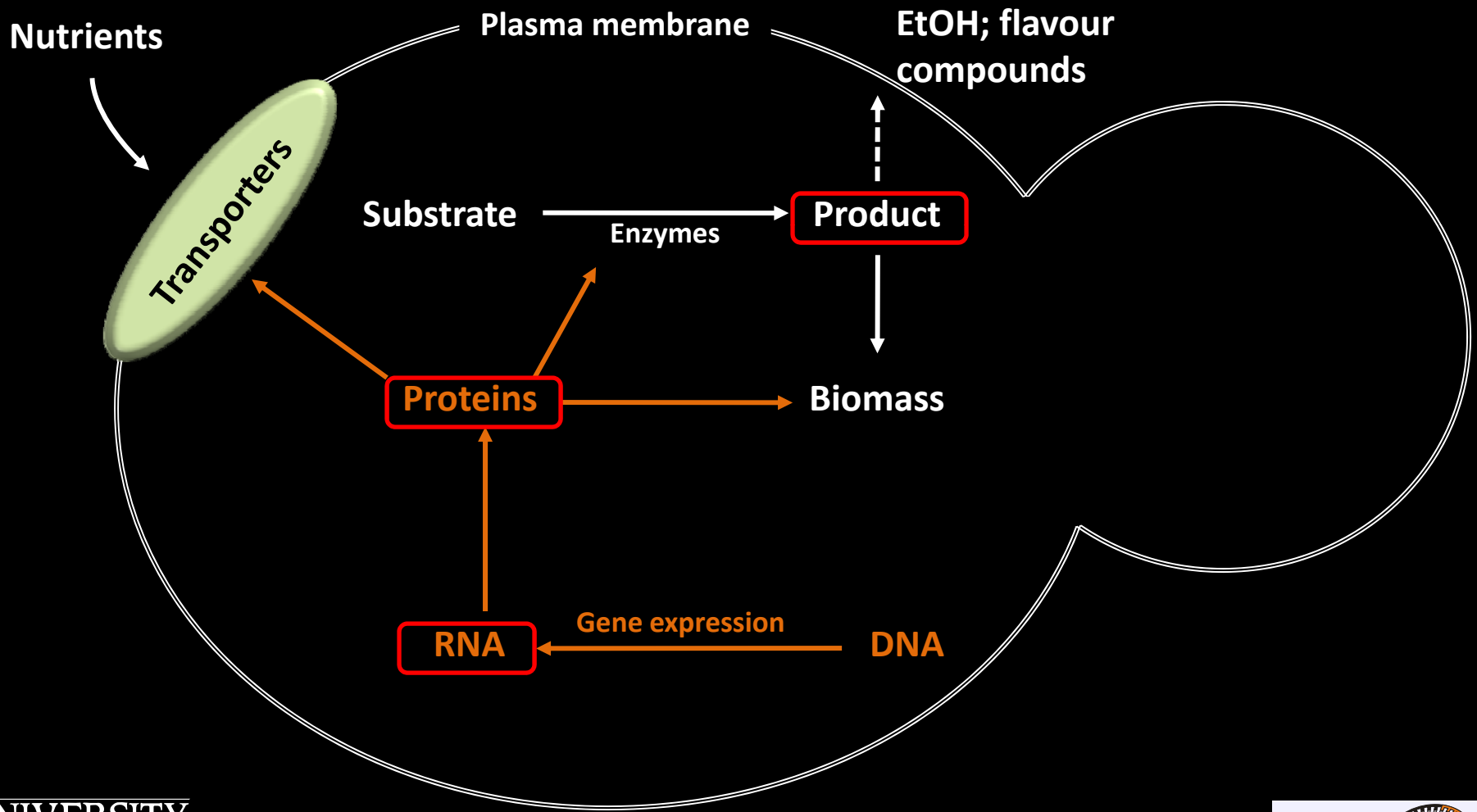
- Inefficient fermentations (stuck/sluggish)
  - Delay in sugar utilization and nutrient uptake; affects product quality
    - Off-flavour production
    - Spoilage organisms
- Winery efficiency
  - Cellar operations suffer; decreased/delayed production

Loss of \$\$\$

# Wine yeasts are products of domestication

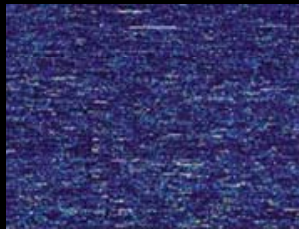
- Several thousand years of wine production
  - Selection of yeast tolerant to wine-making conditions
- Wine yeasts genomes (Bradbury *et al.*, 2005)
  - Generally diploid or aneuploid
  - Often heterozygous
  - Limited homozygosity
- Sequencing of wine yeast genomes (Borneman *et al.*, 2008; Novo *et al.*, 2009)
  - Large conservations of sequence with lab yeast (S288c)
  - Thousands of polymorphisms
  - Industrial yeast have extended genomes (~37 new genes)
    - Lateral gene transfer?

# How do we analyze the fermentation stress response??



# Transcriptomic analysis of yeast fermentation

- Riesling grape juice
  - Juice characteristics
    - 214 g/L sugar
    - YAN: 210 mg N/L
    - pH 3.3
  - Fermentation at 20 °C; inoculum =  $4 \times 10^6$  cells/mL
- Sample collection
  - 0.5 %, 2 %, 3.5 %, 7 % & 10 % (w/v) ethanol produced
  - RNAs extracted and prepared for microarray analyses; Affymetrix arrays



Absolute expression  
value for each gene

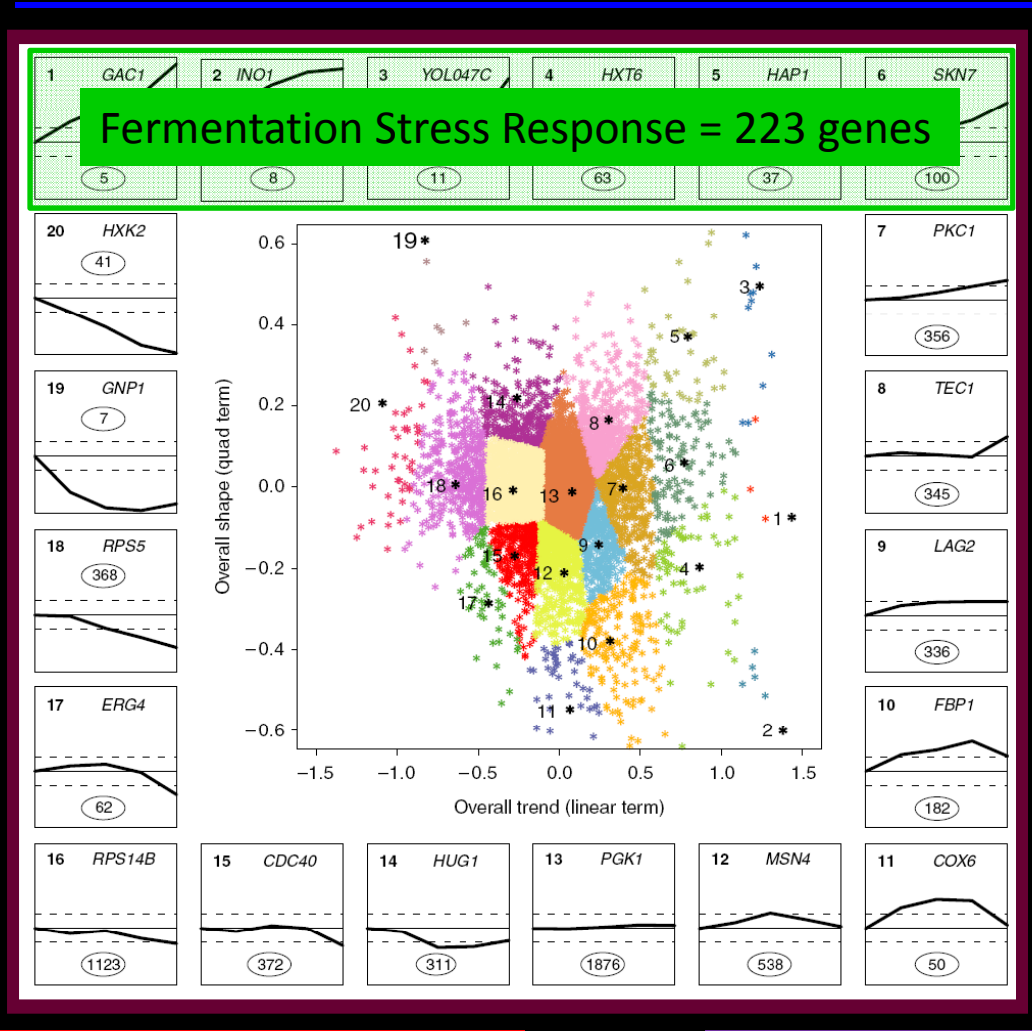
Marks *et al.* (2008)



# Transcriptomic analysis of yeast fermentation

2550 genes had > two-fold change in expression

1279 genes repressed



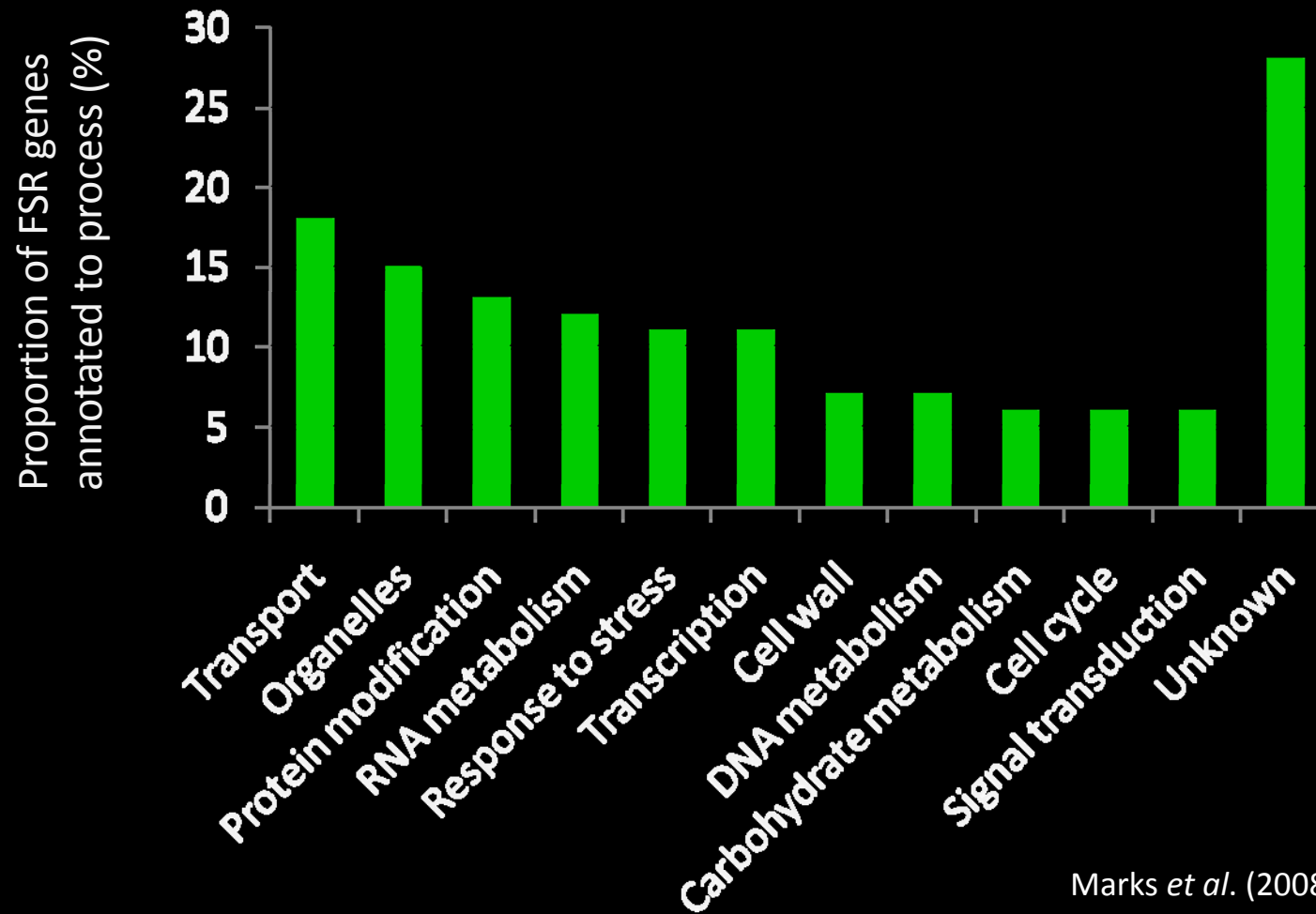
1123 genes induced

148 genes transiently induced

# Transcriptional response: Individual clusters

Cluster	Gene	Fold Change	Biological process	ESR	CER	OS1	OS2	EtOH	N
1	<i>HSP30</i>	80.11	Response to stress				x	x	
2	<i>HSP26</i>	24.45	Response to stress		x	x	x	x	
2	<i>PHM8</i>	28.99	Unknown	x	x	x	x		
3	<i>PES4</i>	21.96	Unknown						
3	<i>ADJ1</i>	20.03	Unknown				x	x	
4	<i>TSL1</i>	8.11	Response to stress	x	x	x	x	x	
4	<i>STR3</i>	12.5	Methionine biosynthesis						
4	<i>JID1</i>	10.41	Unknown						
4	<i>HXT7</i>	9.76	Hexose transport						
4	<i>VID24</i>	6.22	Vesicle-mediated transport						
5	<i>VID27</i>	7.09	Unknown						
5	<i>DIP5</i>	19.61	Amino acid transport						
5	<i>BTN2</i>	12.82	Endosome to Golgi transport		x		x		
6	<i>PMC1</i>	11.16	Calcium ion homeostasis	x	x				
6	<i>XBP1</i>	10.83	Response to stress	x	x		x		
6	<i>YDL010W</i>	9.37	Unknown						
6	<i>YPL230W</i>	7.51	Unknown	x					
6	<i>YAK1</i>	5.51	Protein phosphorylation	x		x	x		x
6	<i>UBC8</i>	5.01	Protein monoubiquitination	x	x		x	x	

# Functional categories of FSR



Marks *et al.* (2008)

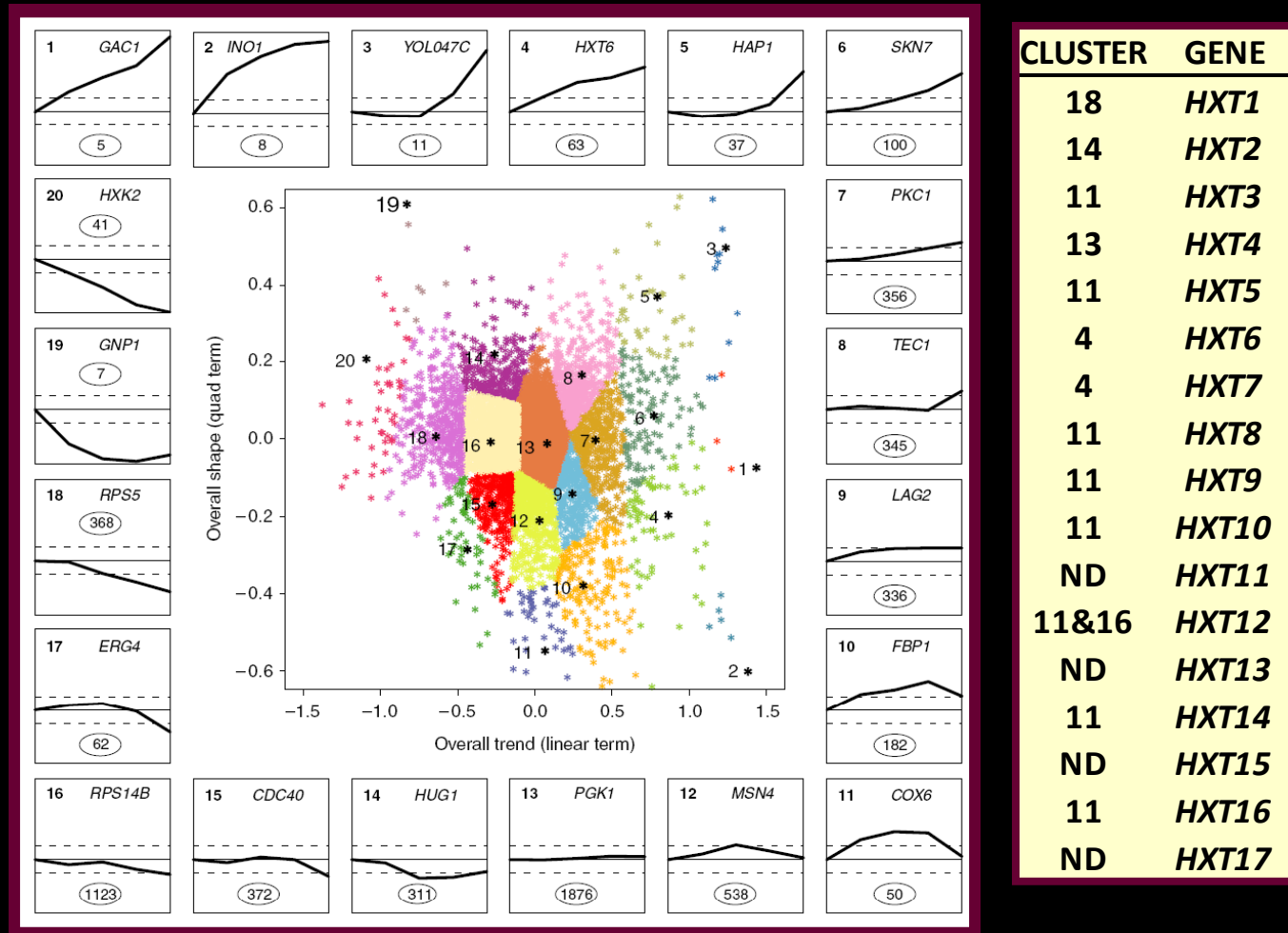
# Transcriptional response: Individual clusters

Cluster	Gene	Fold Change	Biological process	ESR	CER	OS1	OS2	EtOH	N
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5	<i>BTN2</i>	12.82	Endosome to Golgi transport		X		X		
6	<i>PMC1</i>	11.16	Calcium ion homeostasis	X	X				
6	<i>XBP1</i>	10.83	Response to stress	X	X		X		
6	<i>YDL010W</i>	9.37	Unknown						
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6	<i>YAK1</i>	5.51	Protein phosphorylation	X		X	X		X
6	<i>UBC8</i>	5.01	Protein monoubiquitination	X	X		X	X	

# HXT genes: A family of 17 hexose transporters

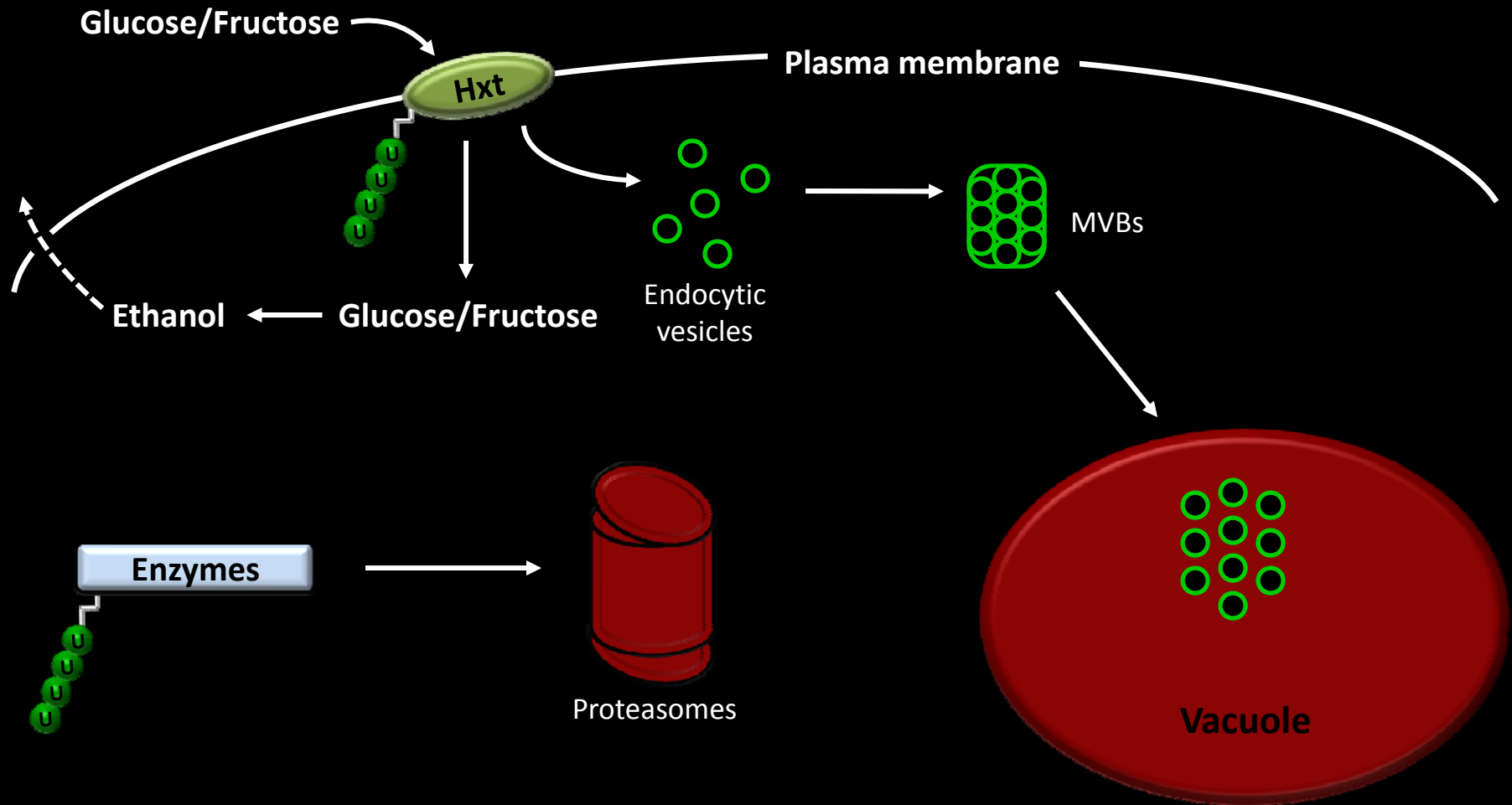
- Hexose transporters import glucose and fructose
  - Low affinity Hxts ( $K_m$  for glucose, ~50-100 mM)
    - Hxt1, Hxt3
  - Moderate affinity Hxts
    - Hxt5
  - High affinity Hxts ( $K_m$  for glucose, ~1-4 mM)
    - Hxt2, Hxt4, Hxt6, Hxt7
- Higher affinity for glucose than fructose
  - 65 mM vs. 125 mM for Hxt3; 2.1 mM vs. 4.6 mM for Hxt7
- Regulation of Hxts in response to nutrients
  - Transcription
  - Protein degradation

# Transcription of *HXT* genes during Riesling fermentation



Marks *et al.* (2008)

# Nutrient-regulated protein turnover

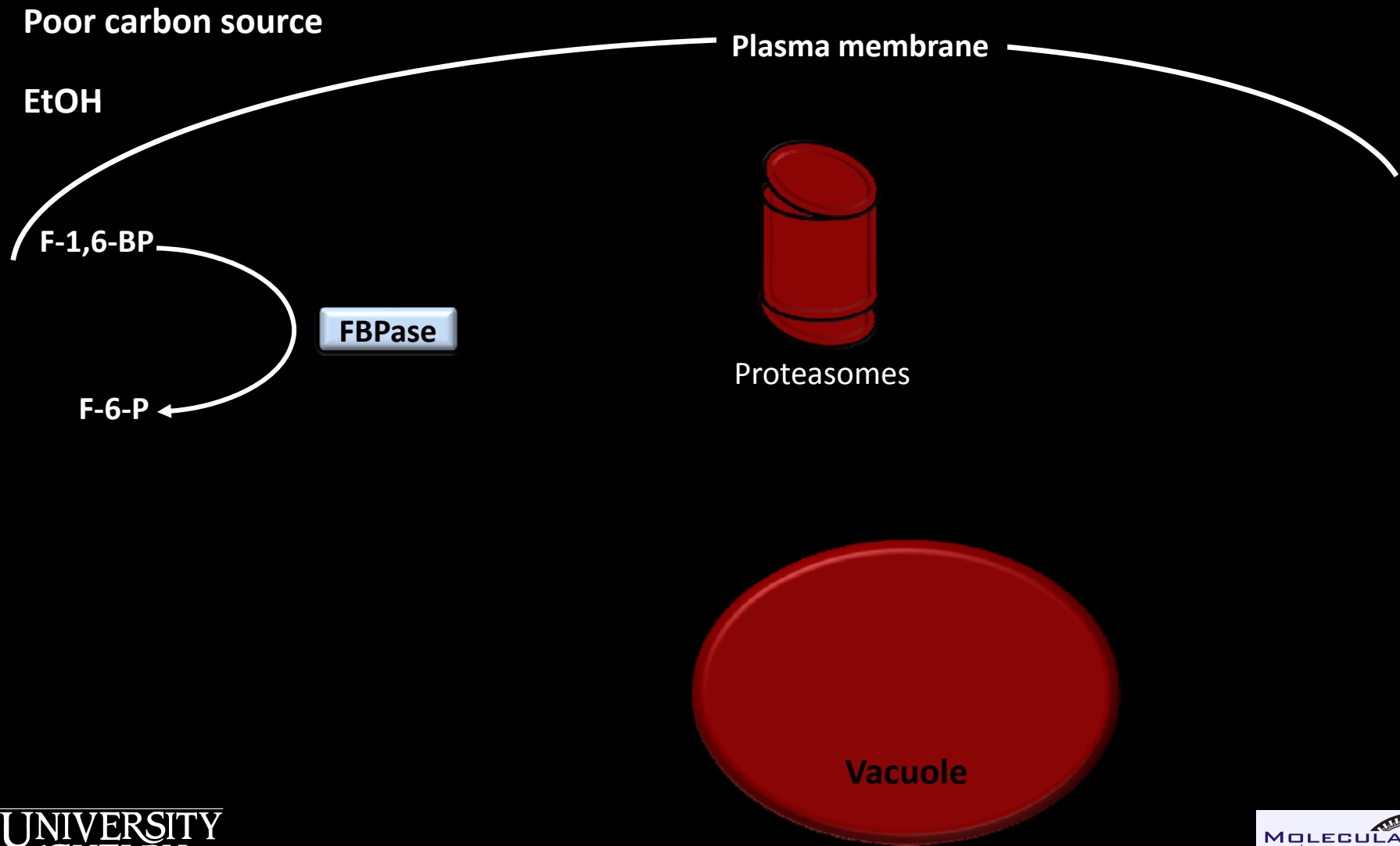


# Transcriptional response: Individual clusters

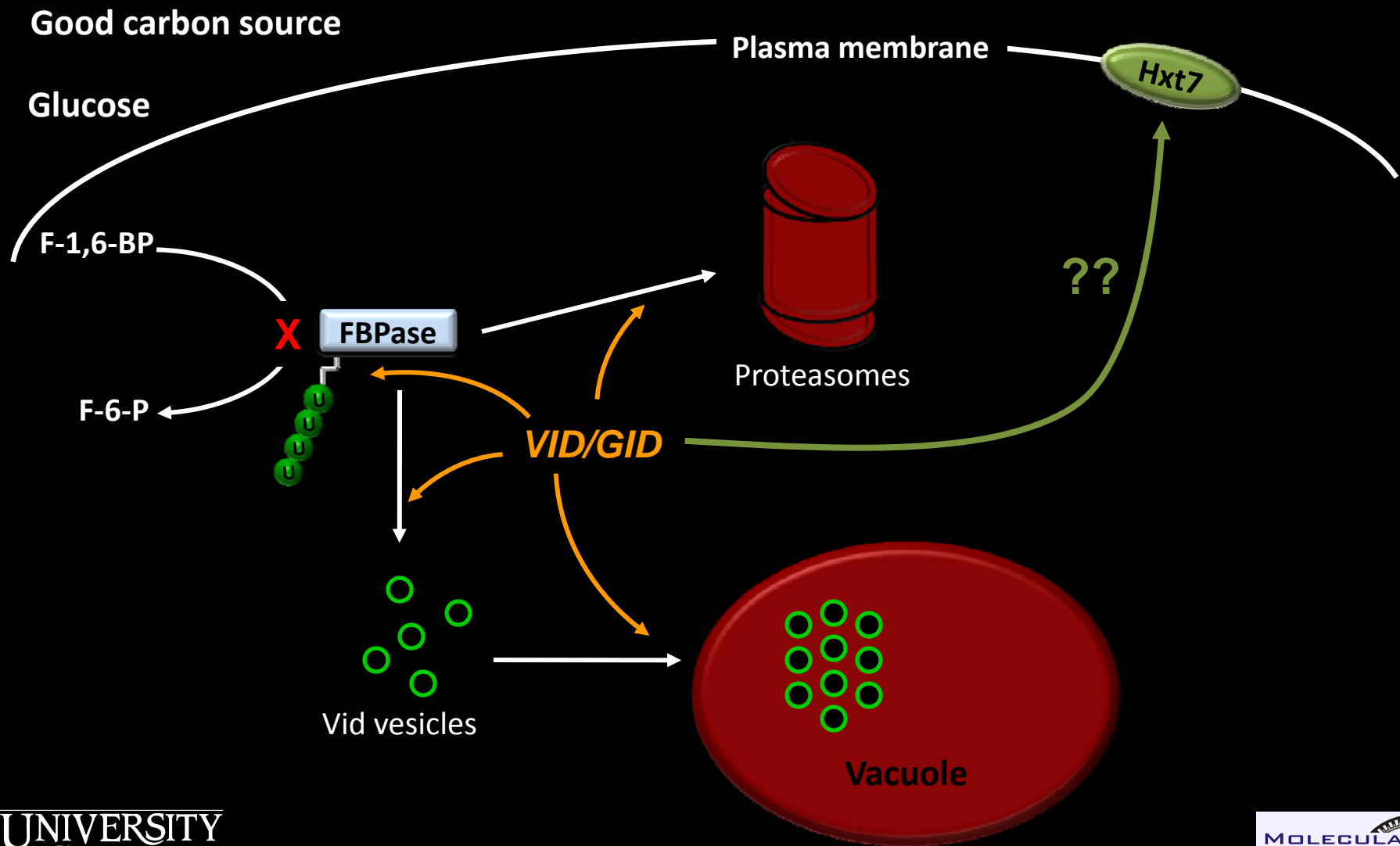
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5	<i>DIP5</i>	19.61	Amino acid transport						
5	<i>BTN2</i>	12.82	Endosome to Golgi transport		X		X		
6	<i>PMC1</i>	11.16	Calcium ion homeostasis	X	X				
6	<i>XBP1</i>	10.83	Response to stress	X	X		X		
6	<i>YDL010W</i>	9.37	Unknown						
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6	<i>YAK1</i>	5.51	Protein phosphorylation	X		X	X		X
6	<i>UBC8</i>	5.01	Protein monoubiquitination	X	X		X	X	



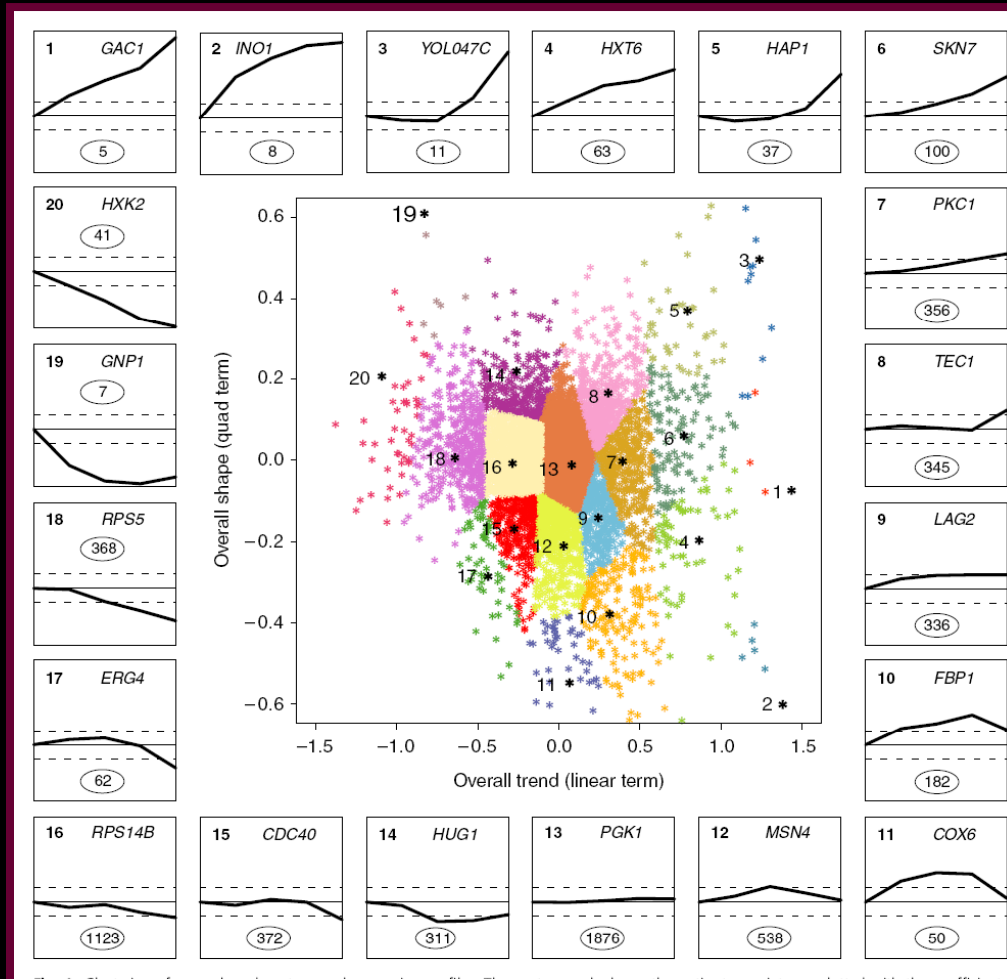
# VIDs & Protein turnover



# VID/GID genes & Protein turnover



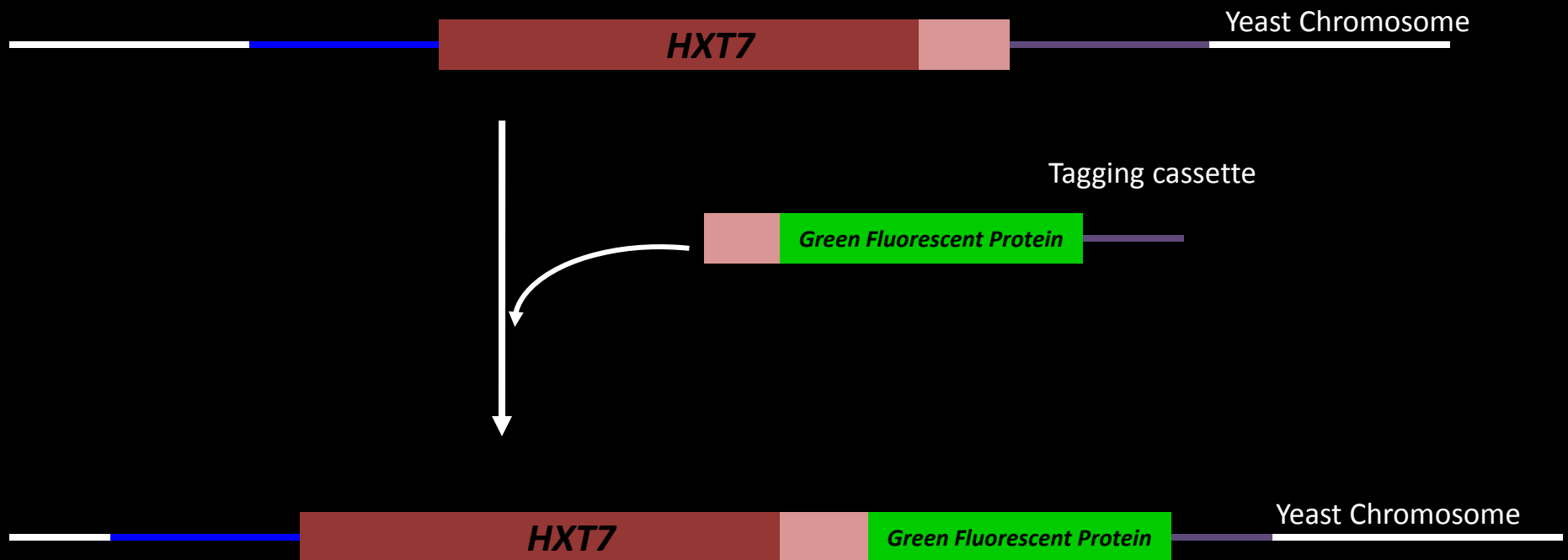
# Transcription of *VID/GID* genes during Riesling fermentation



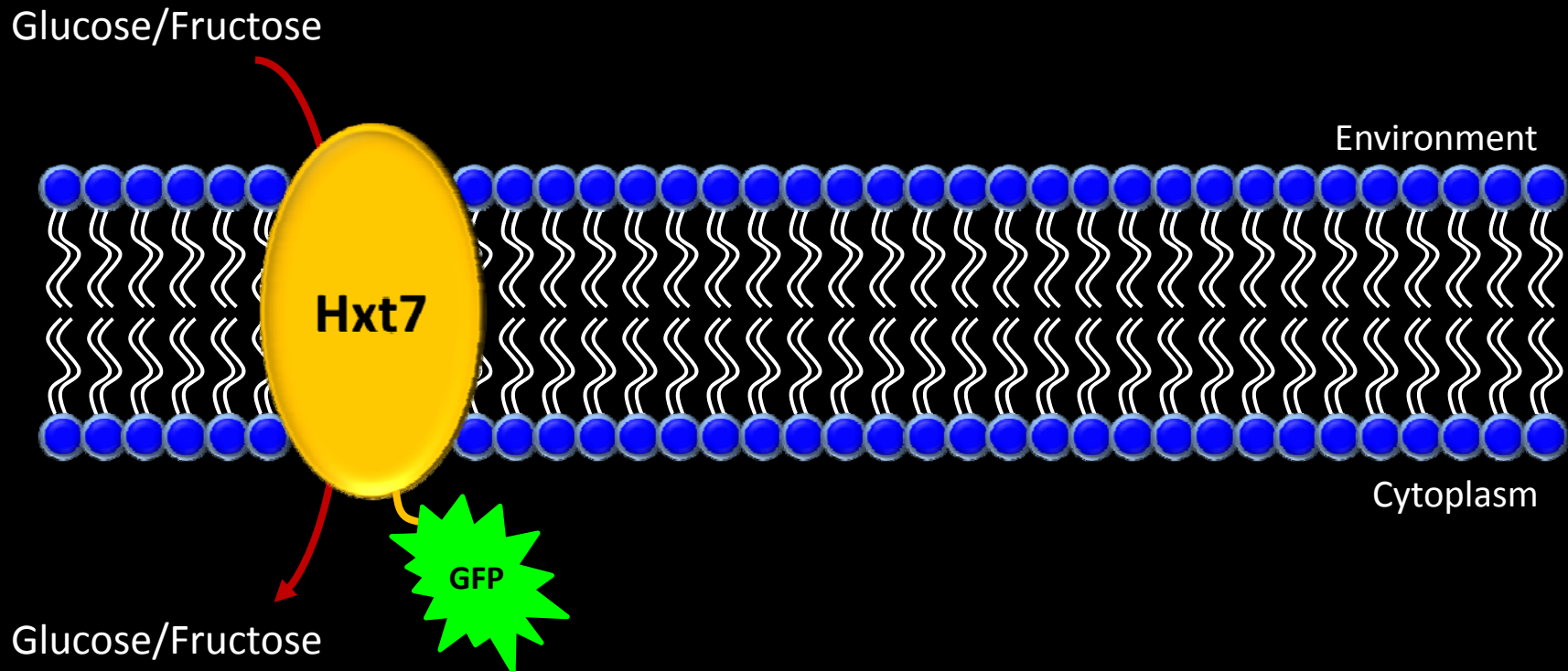
CLUSTER	GENE
10	<i>VID30/GID1</i>
10	<i>GID2</i>
4	<i>VID24/GID4</i>
10	<i>VID28/GID5</i>
10	<i>MOH2/GID7</i>
7	<i>DCR2/GID8</i>
8	<i>FYV10/GID9</i>
11	<i>YDL176W</i>
8	<i>MOH1</i>
6	<i>UBC8/GID3</i>
11	<i>UBP14/GID6</i>
5	<i>VID27</i>
11	<i>VID22</i>
11	<i>ETP1</i>

Marks et al. (2008)

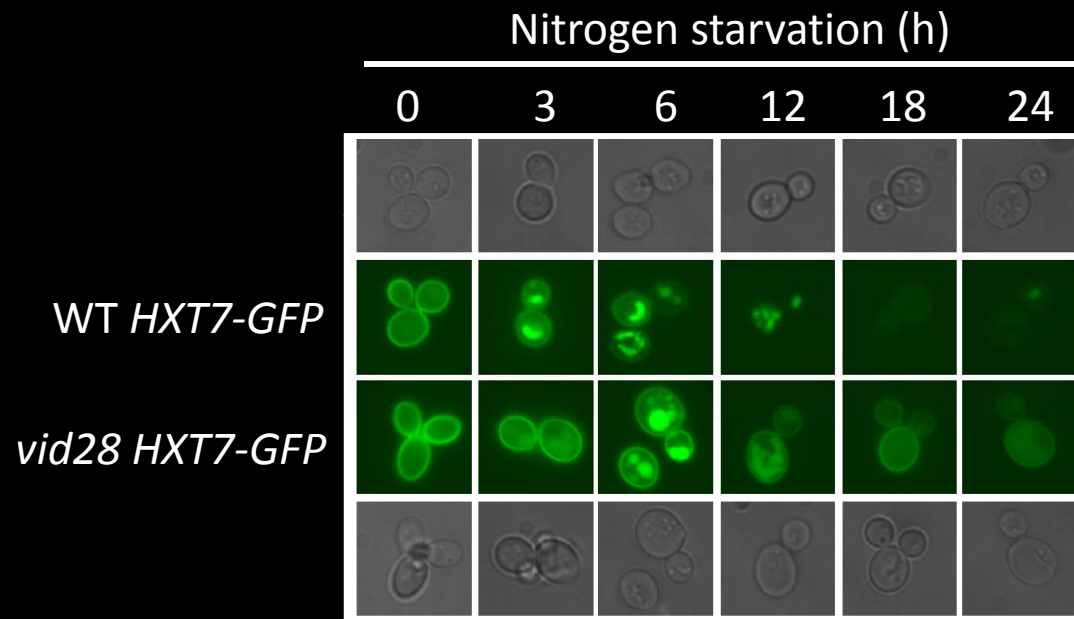
# How can we “see” a protein in a yeast cell?



# GFP-tagged hexose transporter

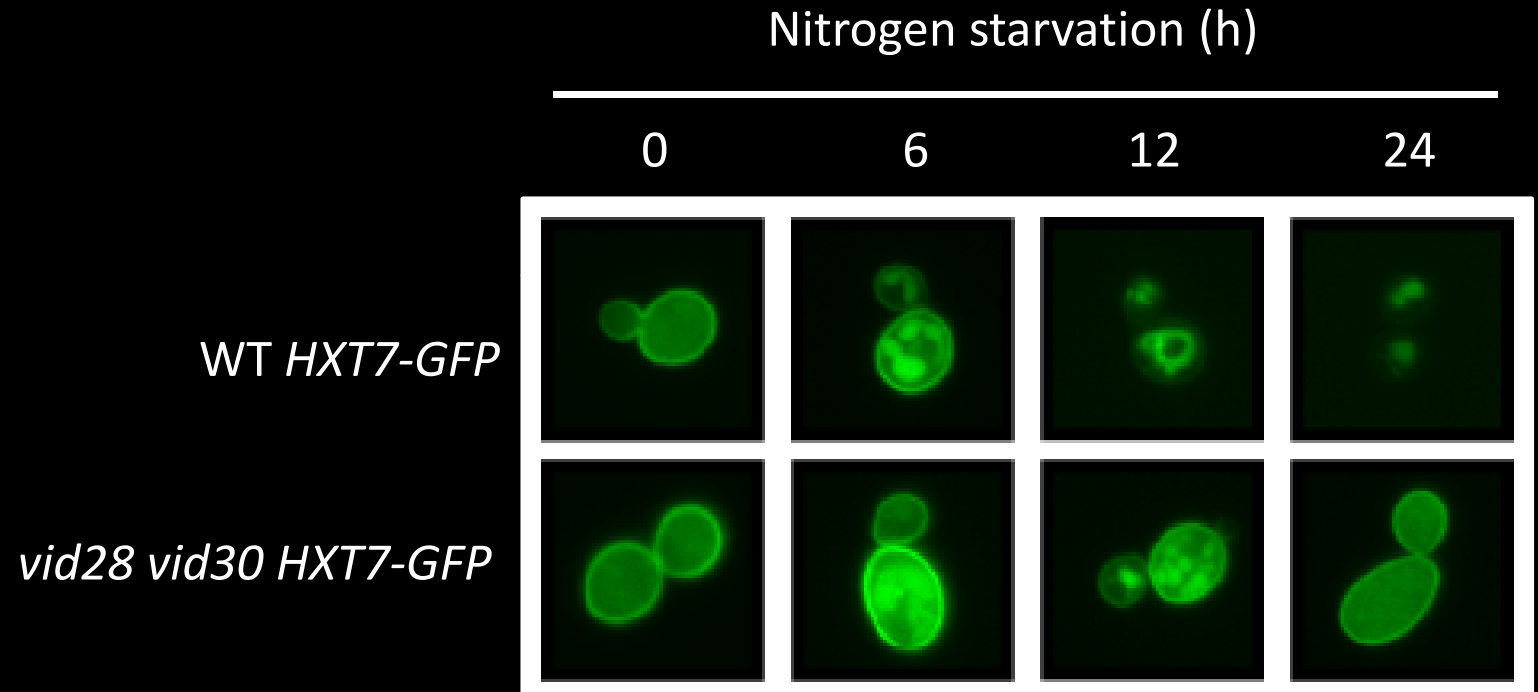


# Vid28 participates in Hxt7 turnover



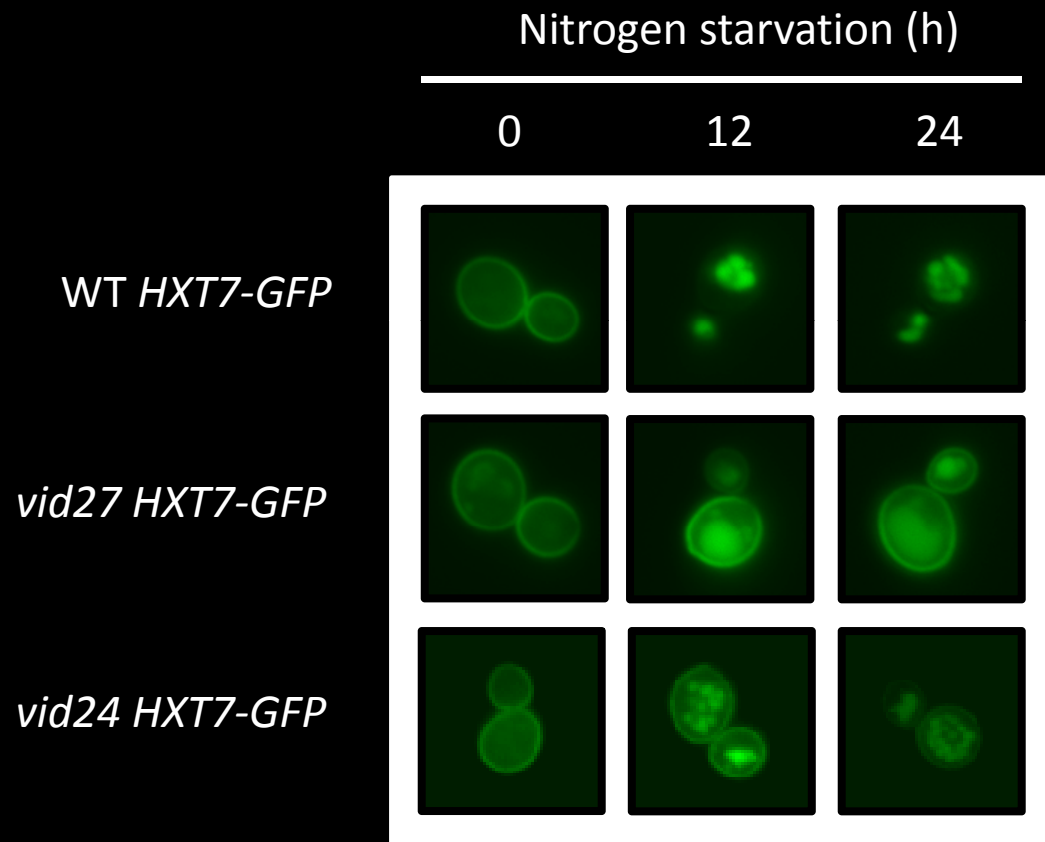
Snowdon *et al.* (2008)

# Role of Vid28 & Vid30 in Hxt7 turnover



Snowdon *et al.* (2008)

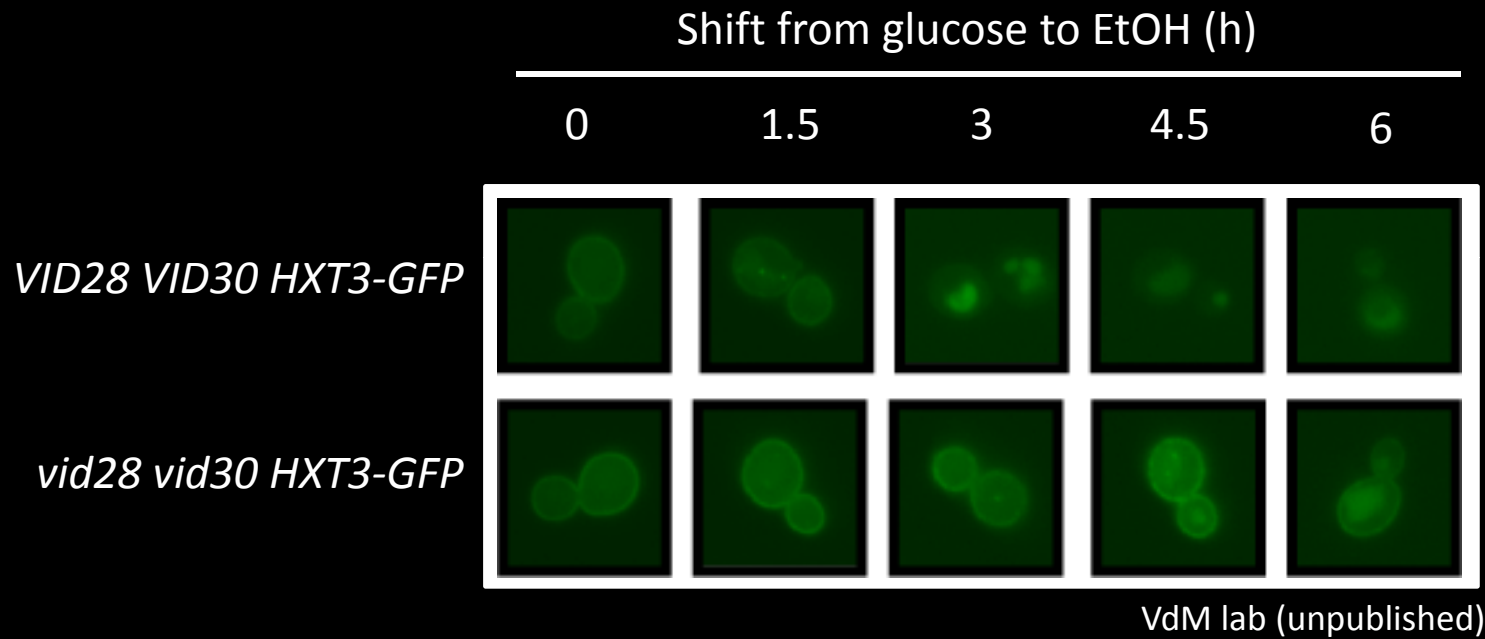
# Vid27 & Vid24 participates in Hxt7 turnover



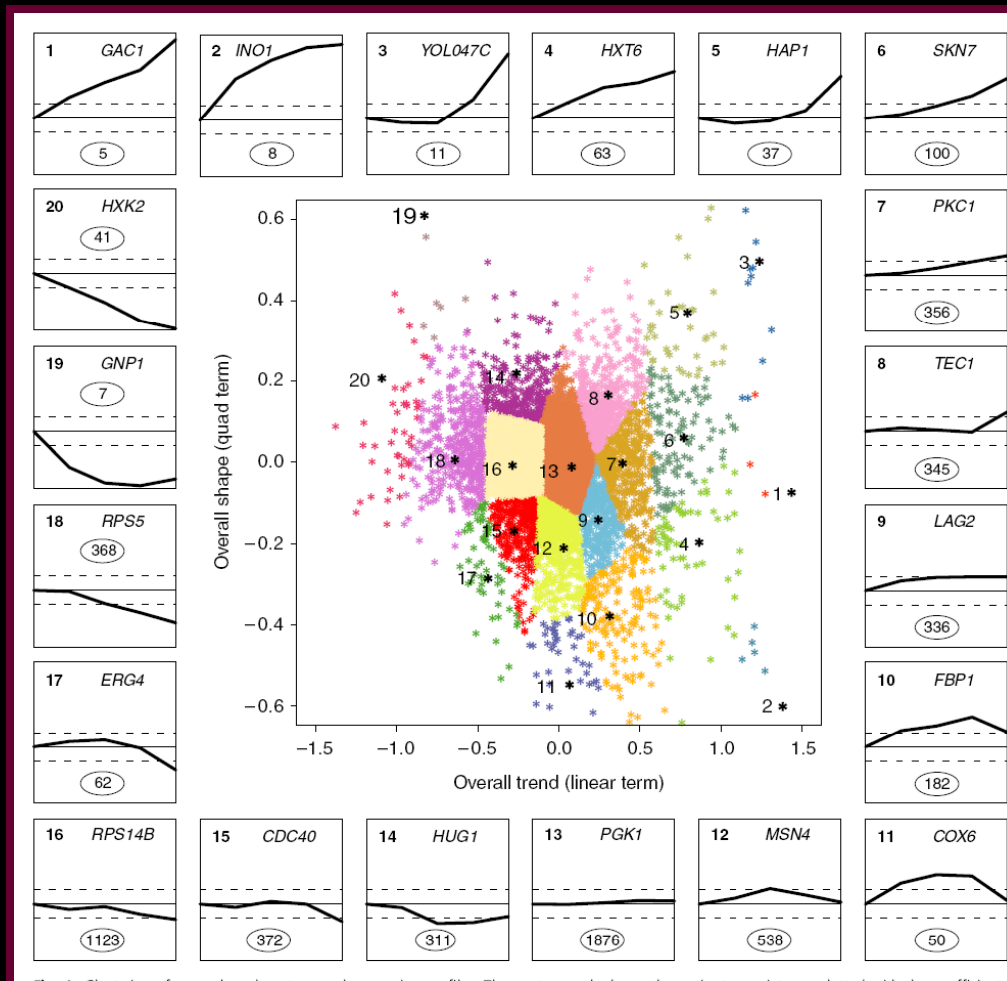
VdM lab (unpublished)



# Vid28 & Vid30 participates in Hxt3 turnover

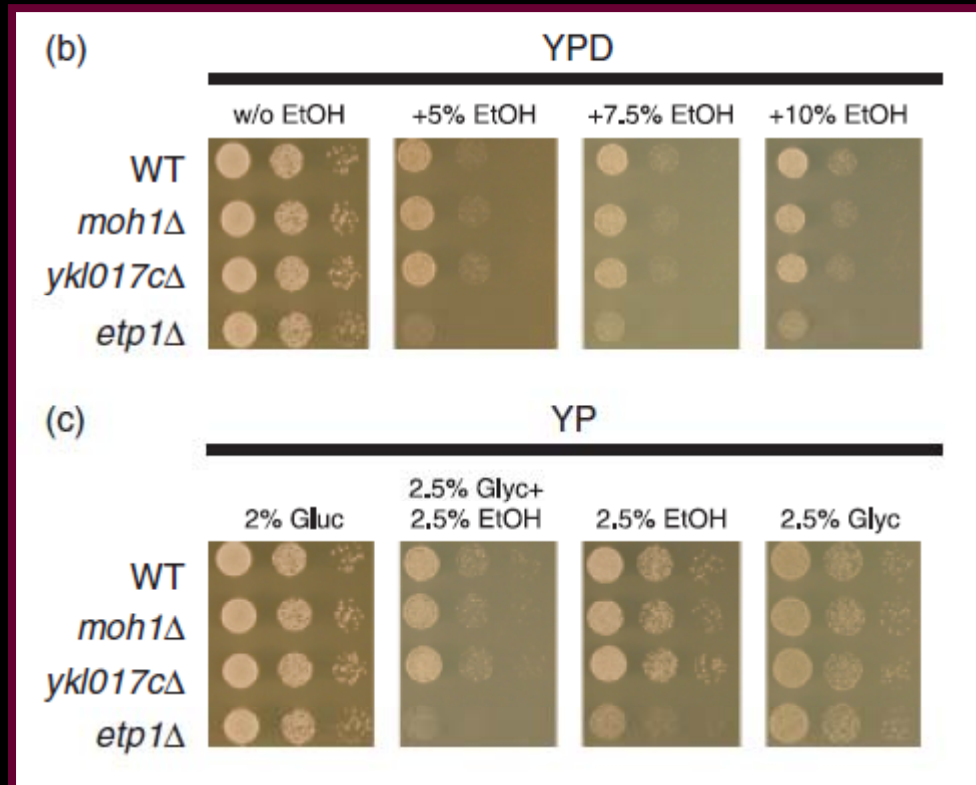


# Etp1 associates with Vids



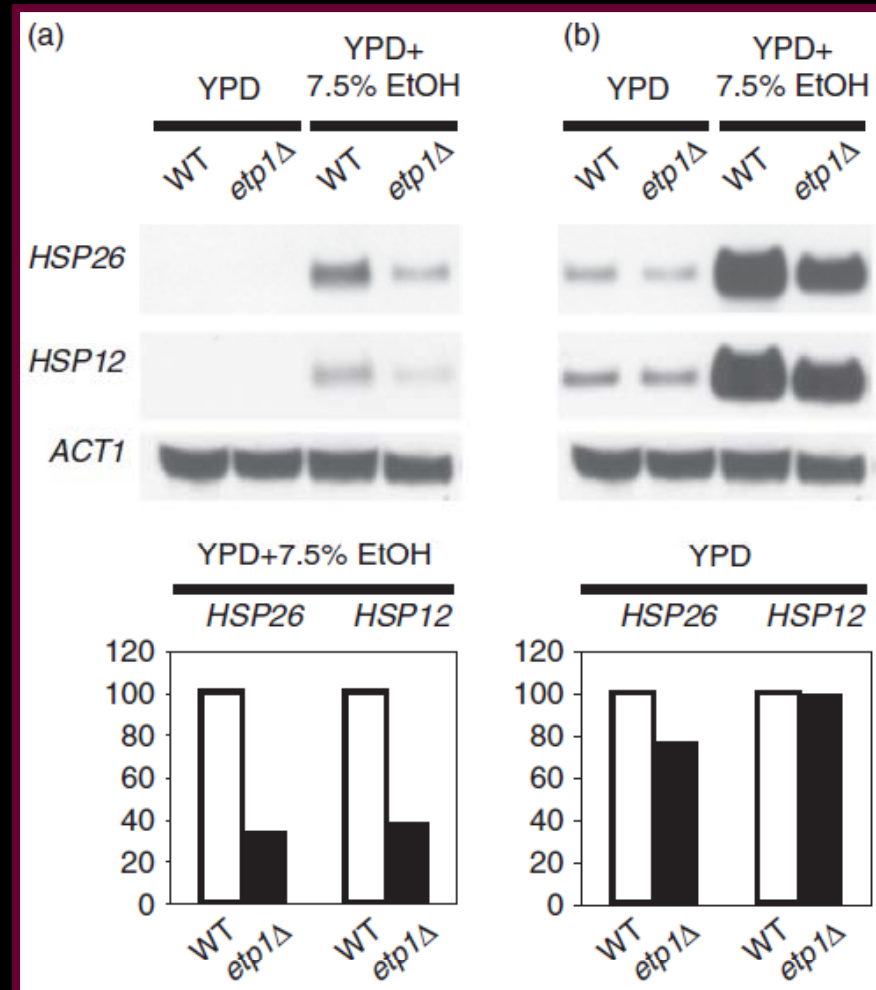
CLUSTER	GENE
10	VID30/GID1
10	GID2
4	VID24/GID4
10	VID28/GID5
10	MOH2/GID7
7	DCR2/GID8
8	FYV10/GID9
11	YDL176W
8	MOH1
6	UBC8/GID3
11	UBP14/GID6
5	VID27
11	VID22
11	<b>ETP1</b>

# Etp1 is needed for ethanol tolerance



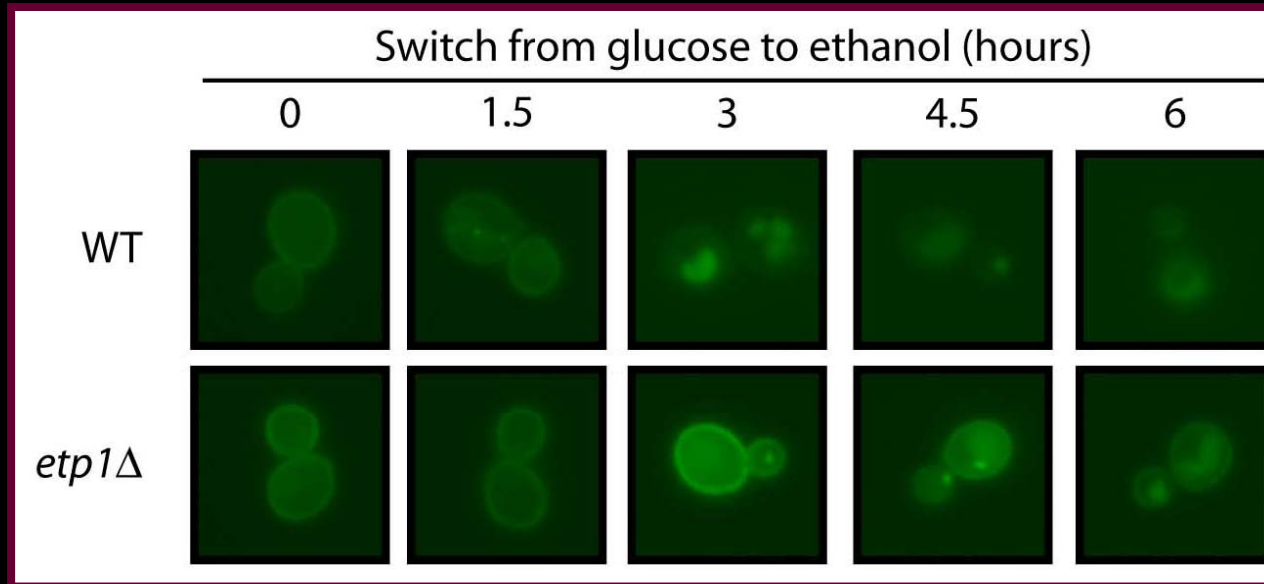
Snowdon *et al.* (2009)

# Etp1 is needed for *HSP* gene activation



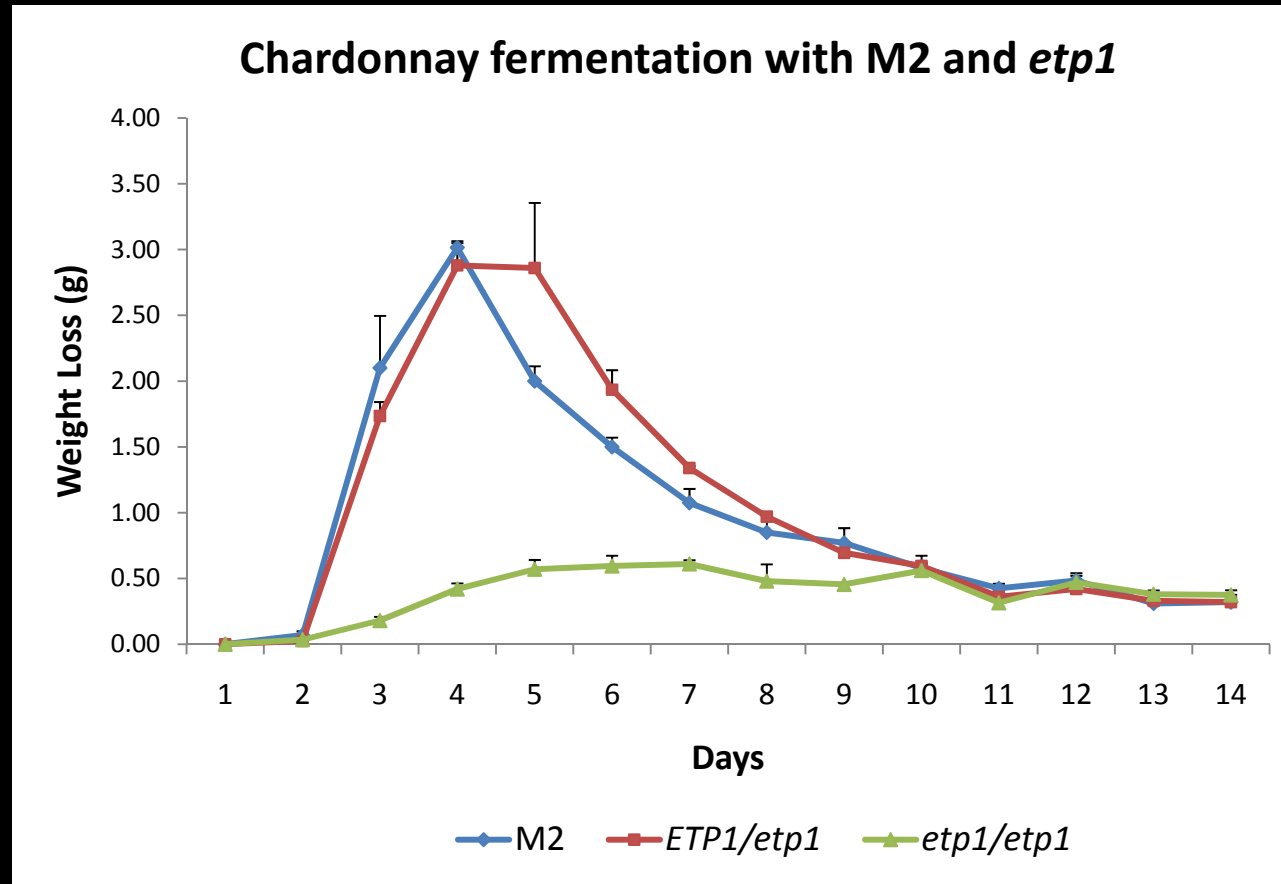
Snowdon *et al.* (2009)

# Etp1 is needed for Hxt3 turnover



Snowdon *et al.* (2009)

# Etp1 is essential for efficient fermentation



# Conclusions & Perspectives

- Functional genomics investigation of vinification revealed FSR genes
  - Integrated response; 60 uncharacterized genes
  - *VID/GID* genes form part of this response
    - Needed for the nutrient-dependent degradation of Hxts
  - *ETP1* – novel gene needed for ethanol tolerance and efficient wine fermentation
- Impact of Vids and Etp1 on metabolome/wine quality?
- Proteomics during vinification to identify:
  - Proteome adaptation
  - More protein targets of Vidproteins
- Data used to develop strategies to overcome fermentation stress

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## GvdM lab members

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**Stephanie Hughes**  
Erik Nielson  
Stephanie Hallows  
**Peter Poliszczuk**  
Lisa Lin

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Hennie van Vuuren (UBC)  
Vivian Measday (UBC)  
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