Alcoholic fermentation

Dr. Nick Coleman

Learning objectives

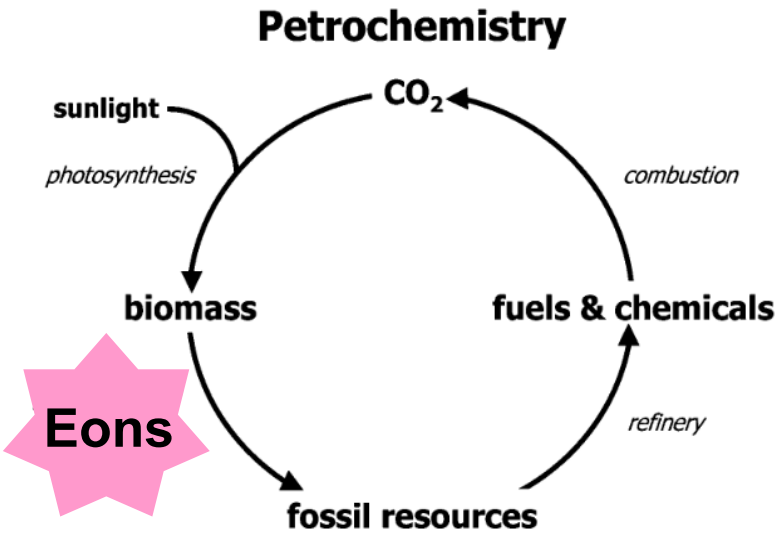
Be able to :

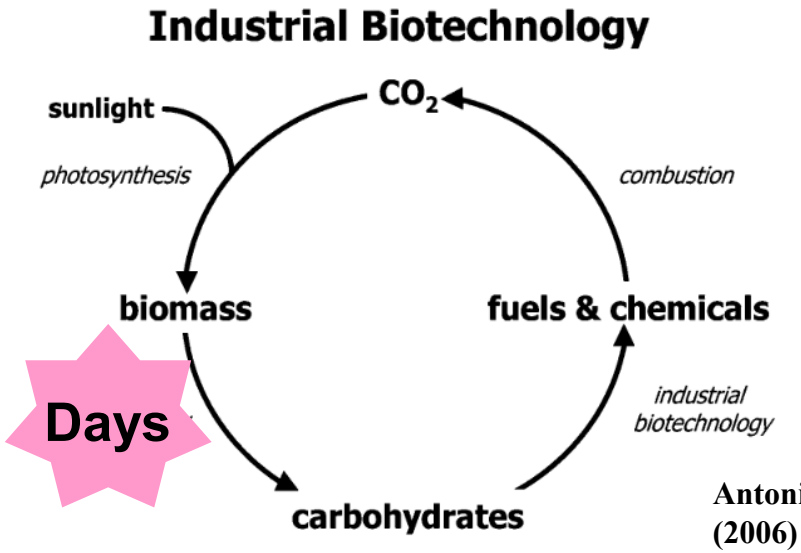
- **Explain the metabolic processes (enzymes, cofactors, metabolites, pathways) involved in alcoholic fermentation**
- **Compare and contrast alcoholic fermentation to other kinds of metabolism (aerobic respiration, other fermentations) – what are the common features, and what are the key differences?**
- **Discuss methods for controlling and optimising alcoholic fermentation, with respect to both yeast biology and also the fermentation environment**

Alcoholic fermentation is important

- **Beverages:** beer, wine, spirits
- **Fuel:** clean-burning, high octane
- **Antimicrobial:** Hand-sanitiser !
- **Solvent:** e.g. ‘methylated spirits’

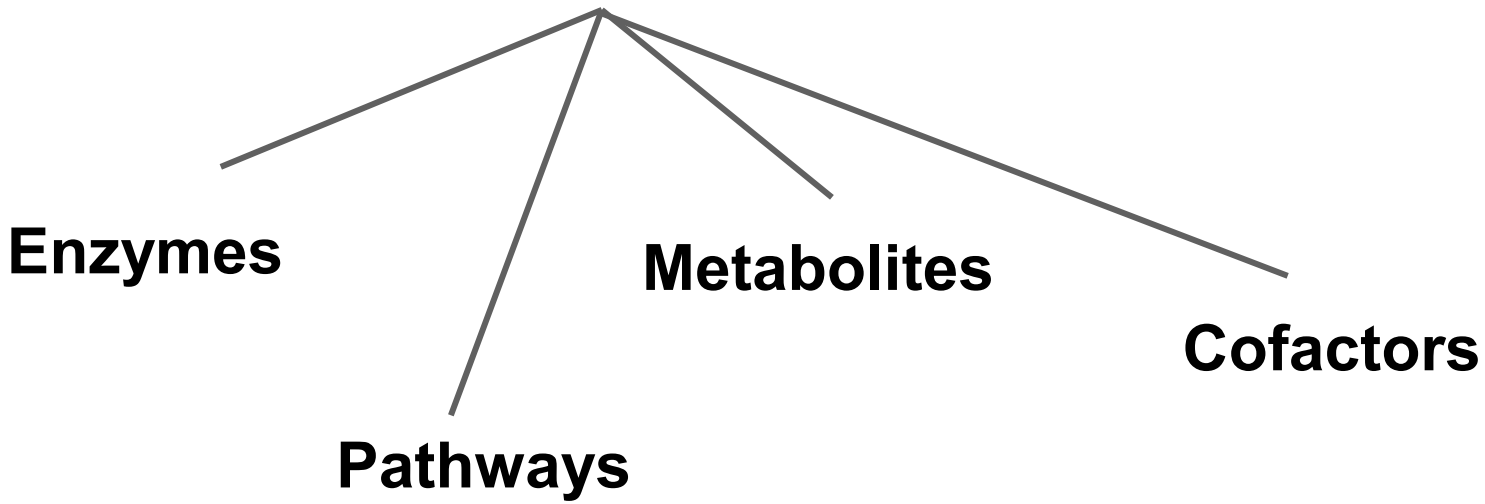
In all cases, ethanol is a RENEWABLE resource





Antonie van Leeuwenhoek (2006) 90:391—418

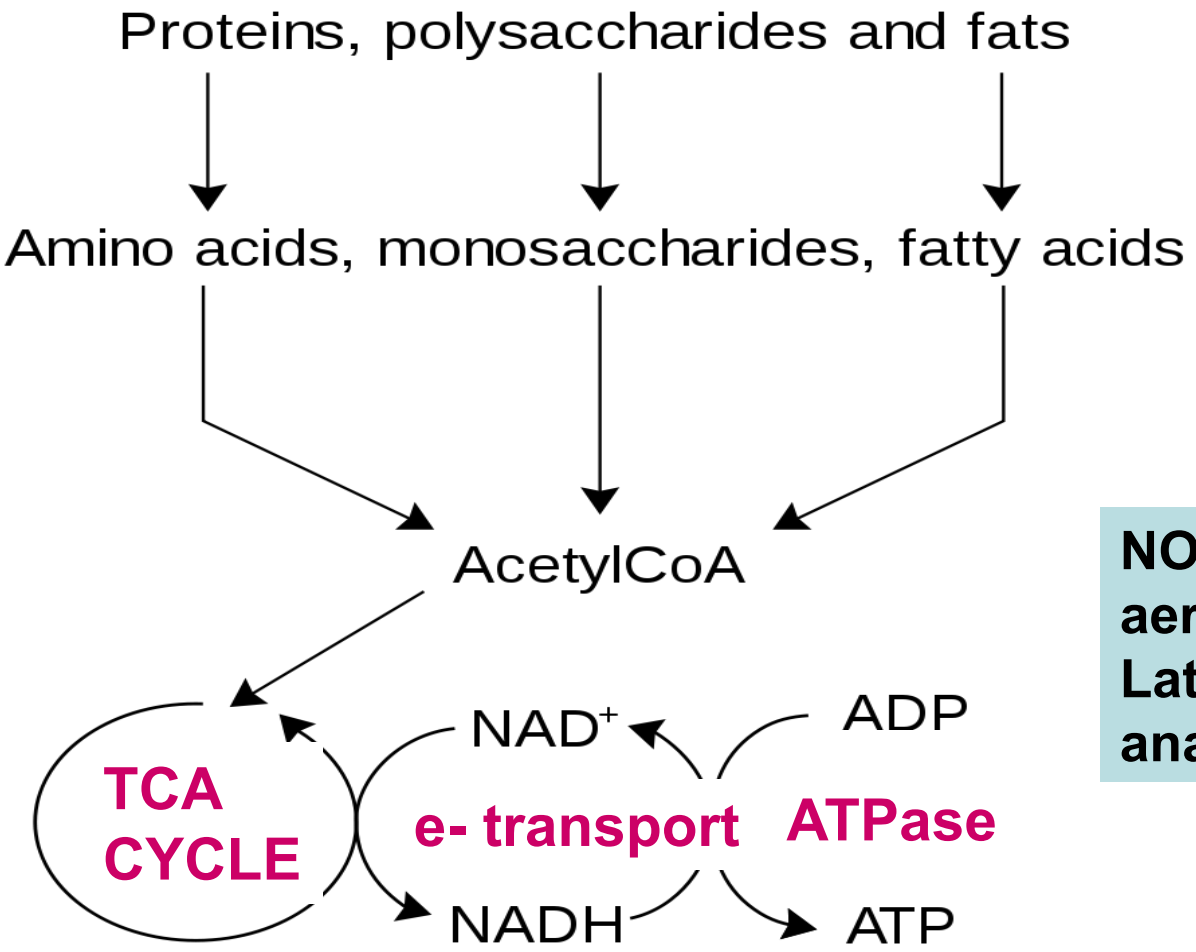
Part 1. Metabolism



Following slides focus on YEAST metabolism (*Saccharomyces cerevisiae*), but many details also true of other microbes

Metabolism: overview

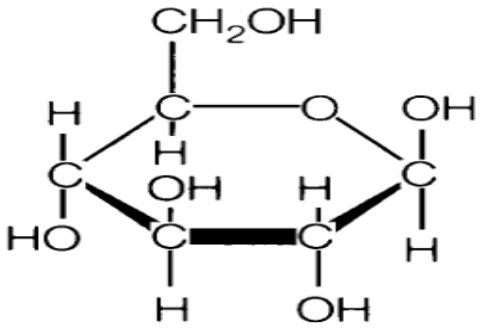
- **Anabolism: building up compounds, energy consumed**
- **Catabolism: breakdown of compounds, energy is made**



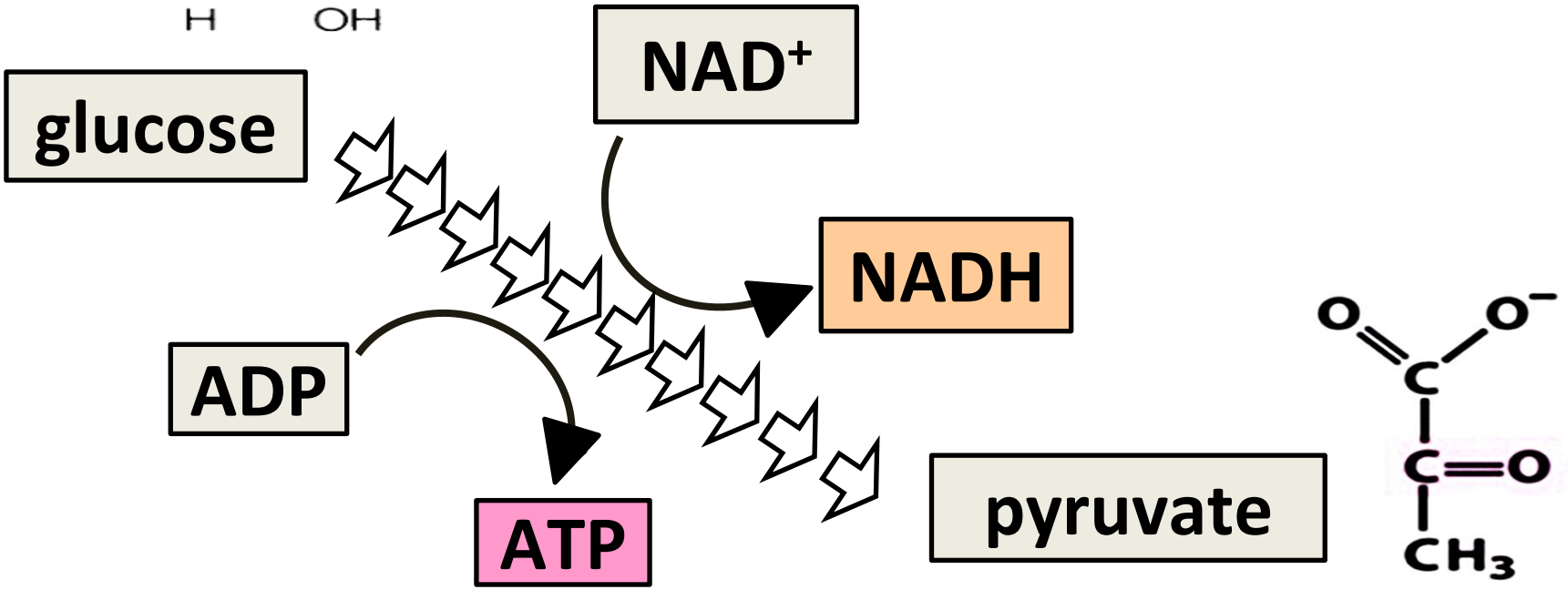
NOTE: This shows aerobic metabolism. Later steps different for anaerobic metabolism !

Metabolism: glycolysis

- Ten enzymes convert glucose → pyruvate
- Energy is captured as ATP and NADH

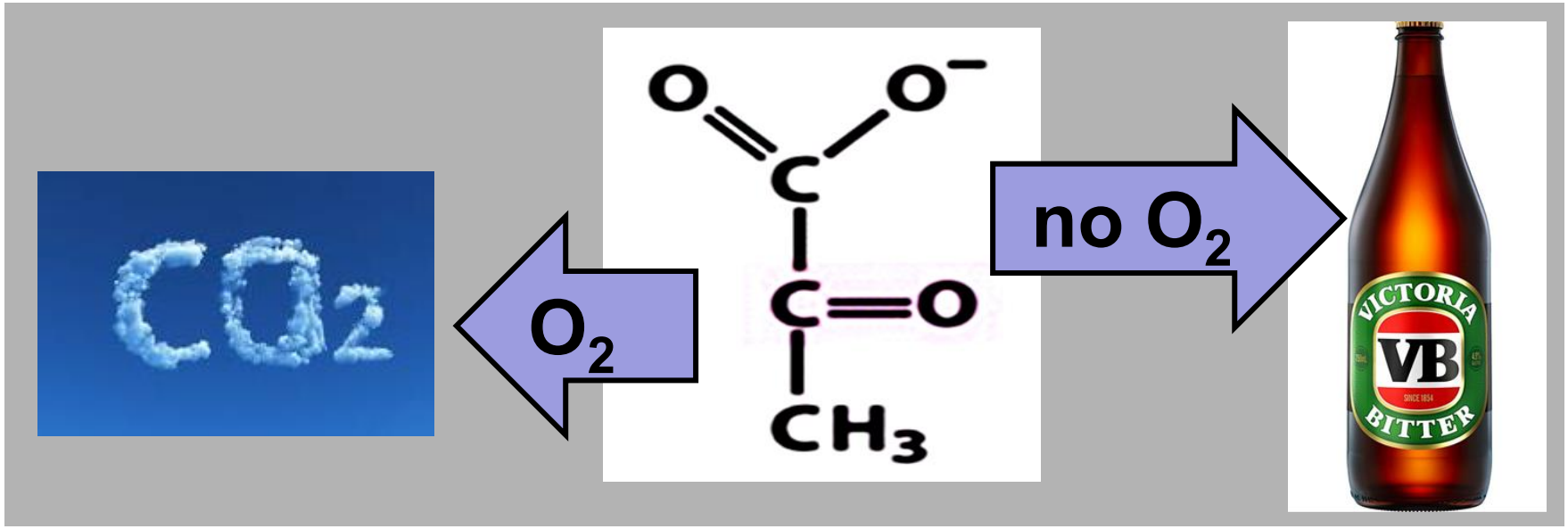


- Cofactors must be regenerated for glycolysis to continue



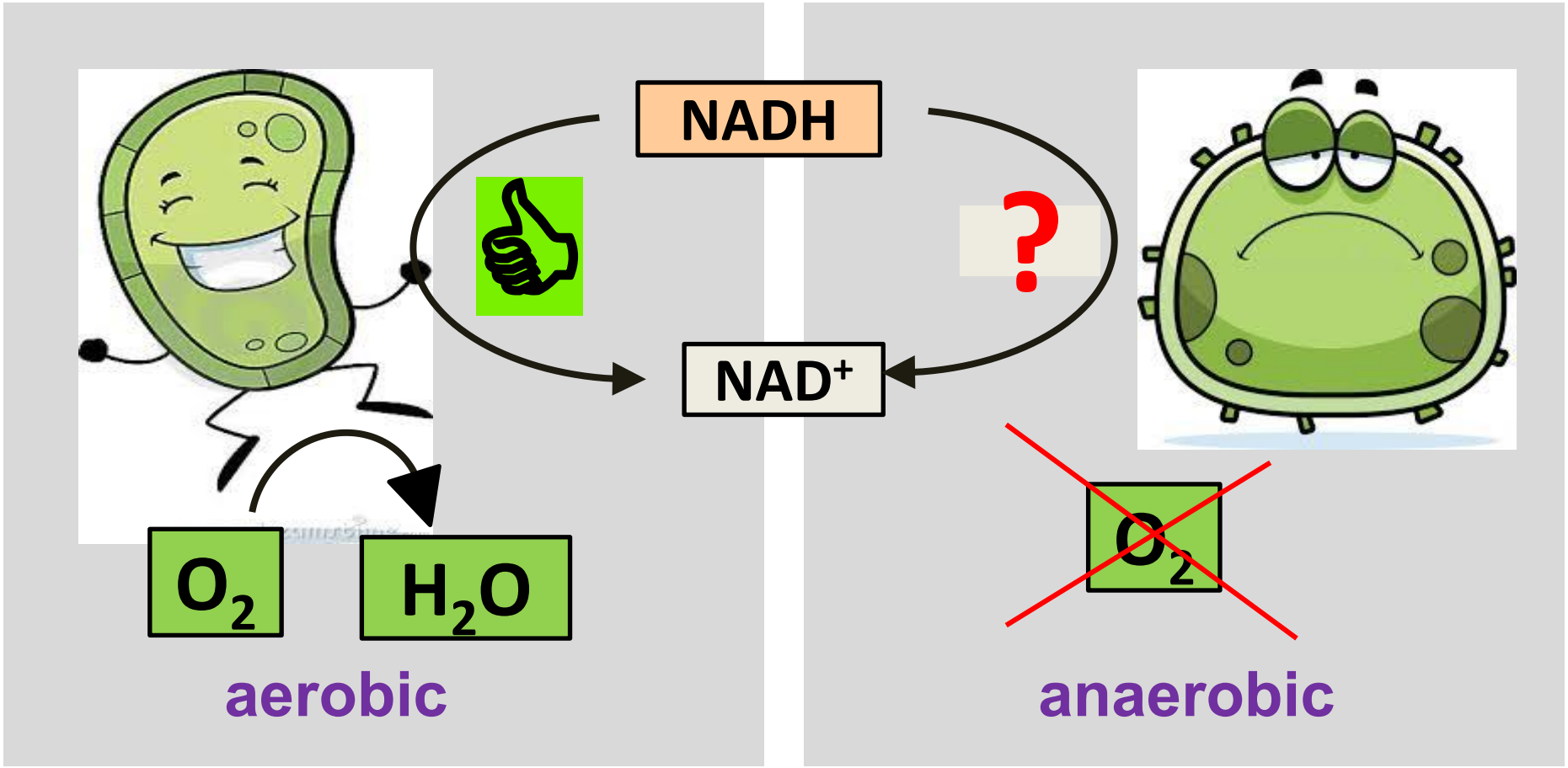
Metabolism: Pyruvate is a key intermediate

- Pyruvate is a major branch point between aerobic and anaerobic metabolic pathways
- Aerobic: pyruvate is oxidised to CO_2 via TCA cycle, electron transport chain, and aerobic respiration
- Anaerobic: pyruvate is fermented to alcohols, acids, H_2



Metabolism: anaerobic conditions?

- Under anaerobic conditions, electron transport chain fails, NADH accumulates, TCA cycle & glycolysis stop

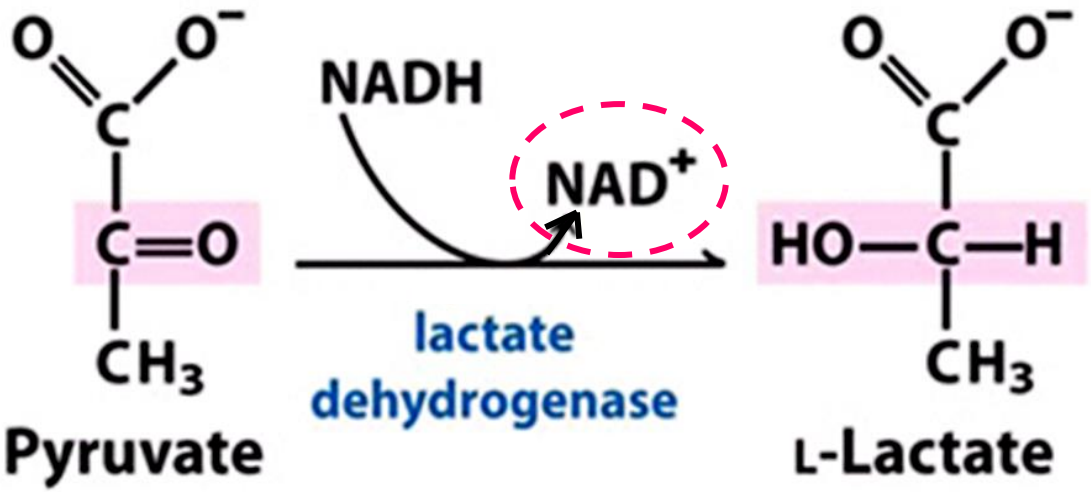


How to regenerate NAD^+ without oxygen?

Use <u>internal</u> e- acceptor eg. pyruvate = <u>fermentation</u>

Types of fermentation. 1. Lactic

- Pyruvate reduced to lactate by lactate dehydrogenase, regenerating NAD+ in the process
- This occurs in many organisms, including humans

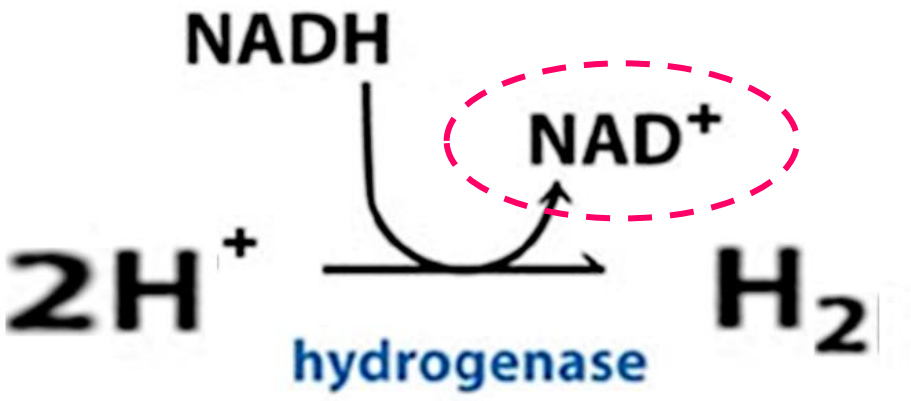


Pain of exercise is partly due to lactate build-up in muscle tissue

Types of fermentation. 2. Hydrogen

- Many microbes contain hydrogenase enzymes that make hydrogen gas via reduction of protons using NADH

- Hydrogenases are reversible, acting as "valves" to balance the ratios of NADH and NAD+ in the cell

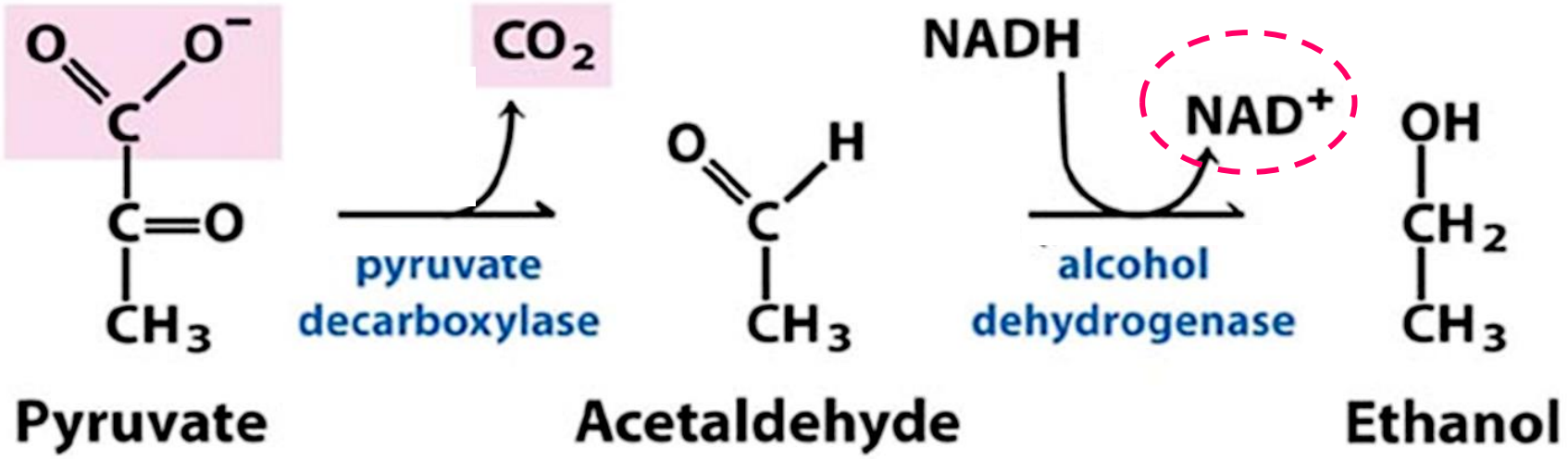


Hydrogen gas is an AWESOME fuel: renewable, clean-burning, powerful

Types of fermentation. 3. Alcoholic

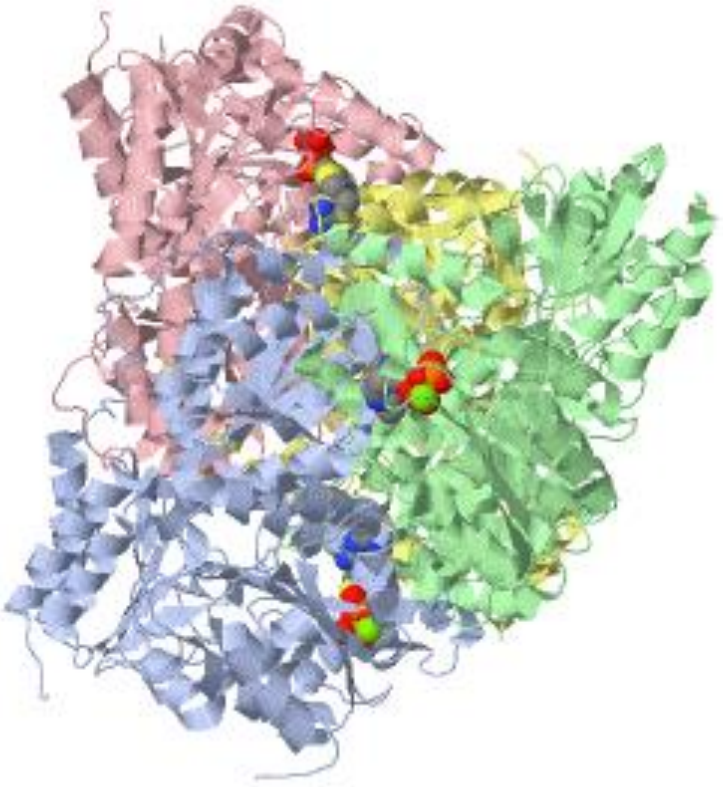
- Pyruvate is cleaved, releasing CO_2, then acetaldehyde is reduced to ethanol, and NAD+ is regenerated.

- Reaction occurs in yeast and in some bacteria



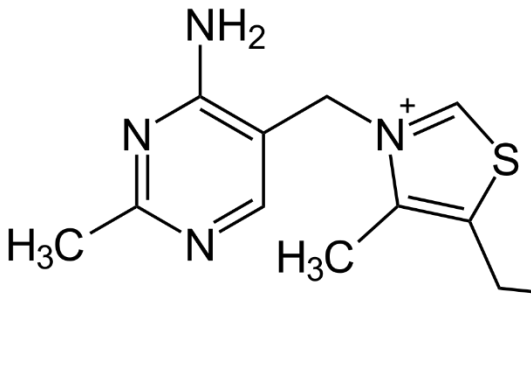
Alcoholic fermentation: enzymes and reactions

Pyruvate decarboxylase enzyme



- **Tetrameric enzyme made of four identical monomers**
- **Requires Mg^{2+} and thiamine* (vitamin B1) as cofactors**

*** Actually thiamine pyrophosphate (TPP)**

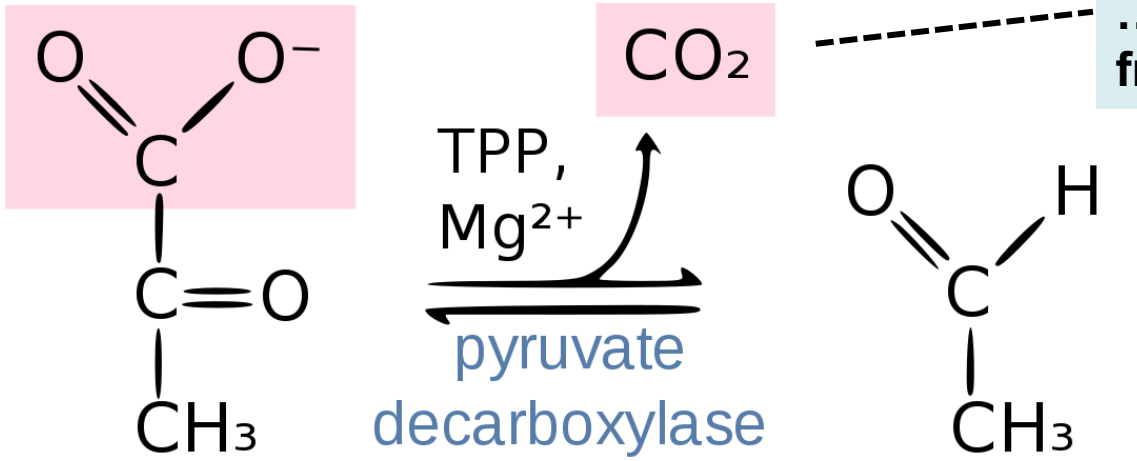


Thiamine

Thiamine - made by microbes + plants, but not by animals or humans
- cofactor for many important catabolic enzymes

Alcoholic fermentation: enzymes and reactions

Pyruvate decarboxylase reaction



Anaerobes also make CO_2 … it doesn't only arise from aerobic respiration

Pyruvate

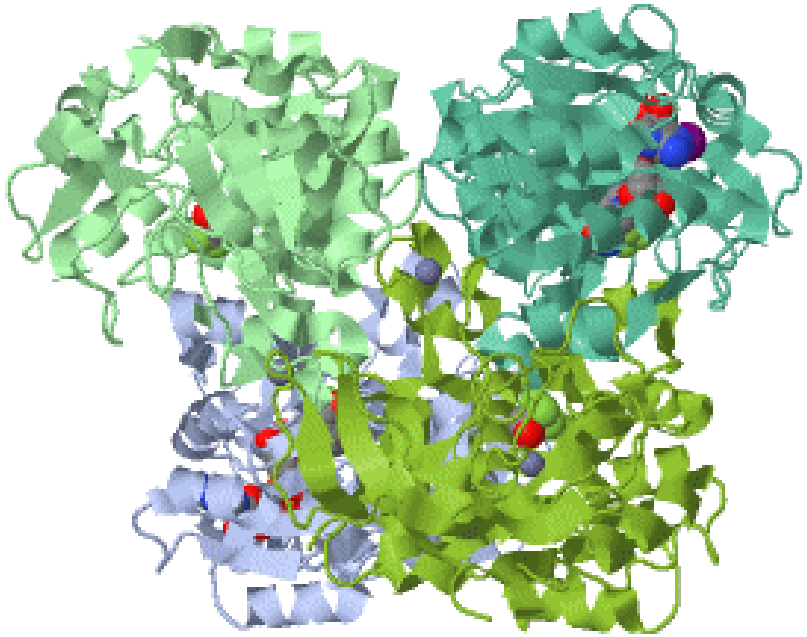
Acetaldehyde

Remember that many other competing reactions can also happen to pyruvate

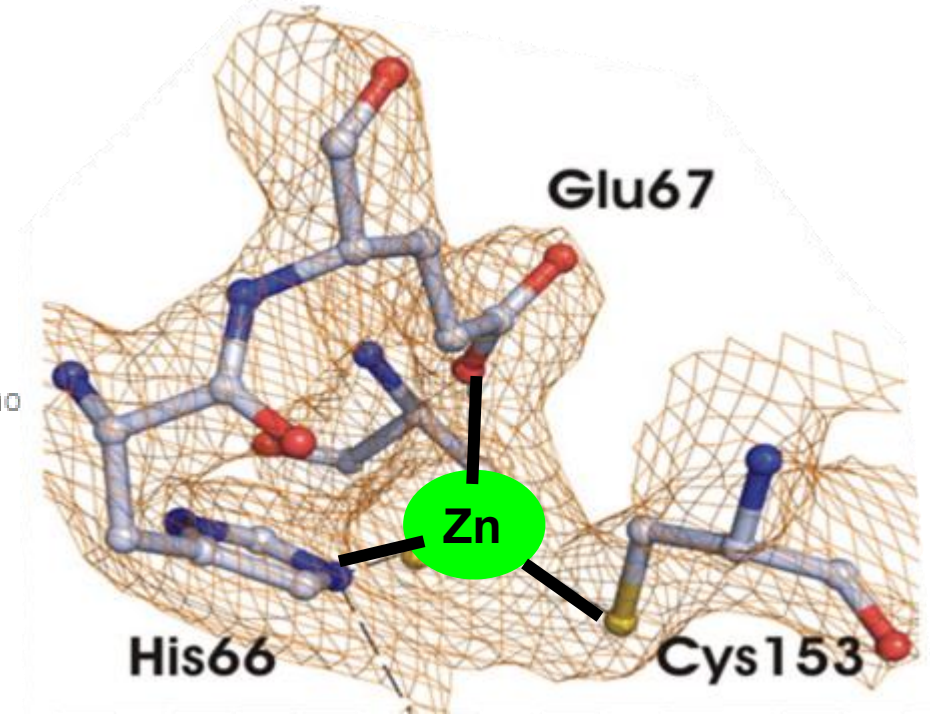
This is TOXIC to all cells and organisms
How to ensure this doesn't accumulate ?
→ need to balance enzyme activities

Alcoholic fermentation: enzymes and reactions

Alcohol dehydrogenase enzyme



- Tetrameric enzyme made of four identical monomers
- Requires zinc (Zn^{2+}) and NAD+ as cofactors



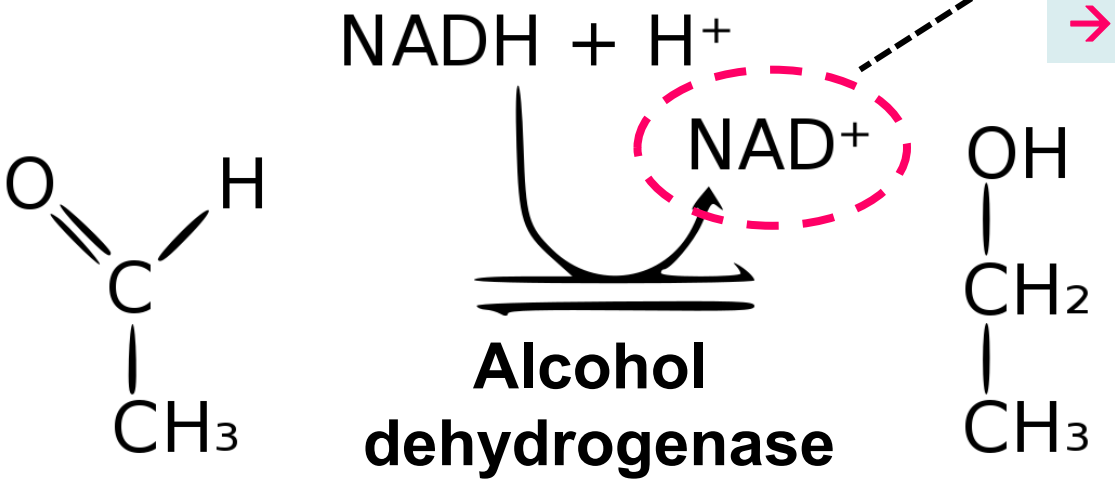
- Negatively charged and/or electron-rich amino acids are important in binding metal cofactors like Zn^{2+}

Jmo

Alcoholic fermentation: enzymes and reactions

Alcohol dehydrogenase reaction

This is **WHY** the yeast cell does this reaction…
→ to replenish NAD+



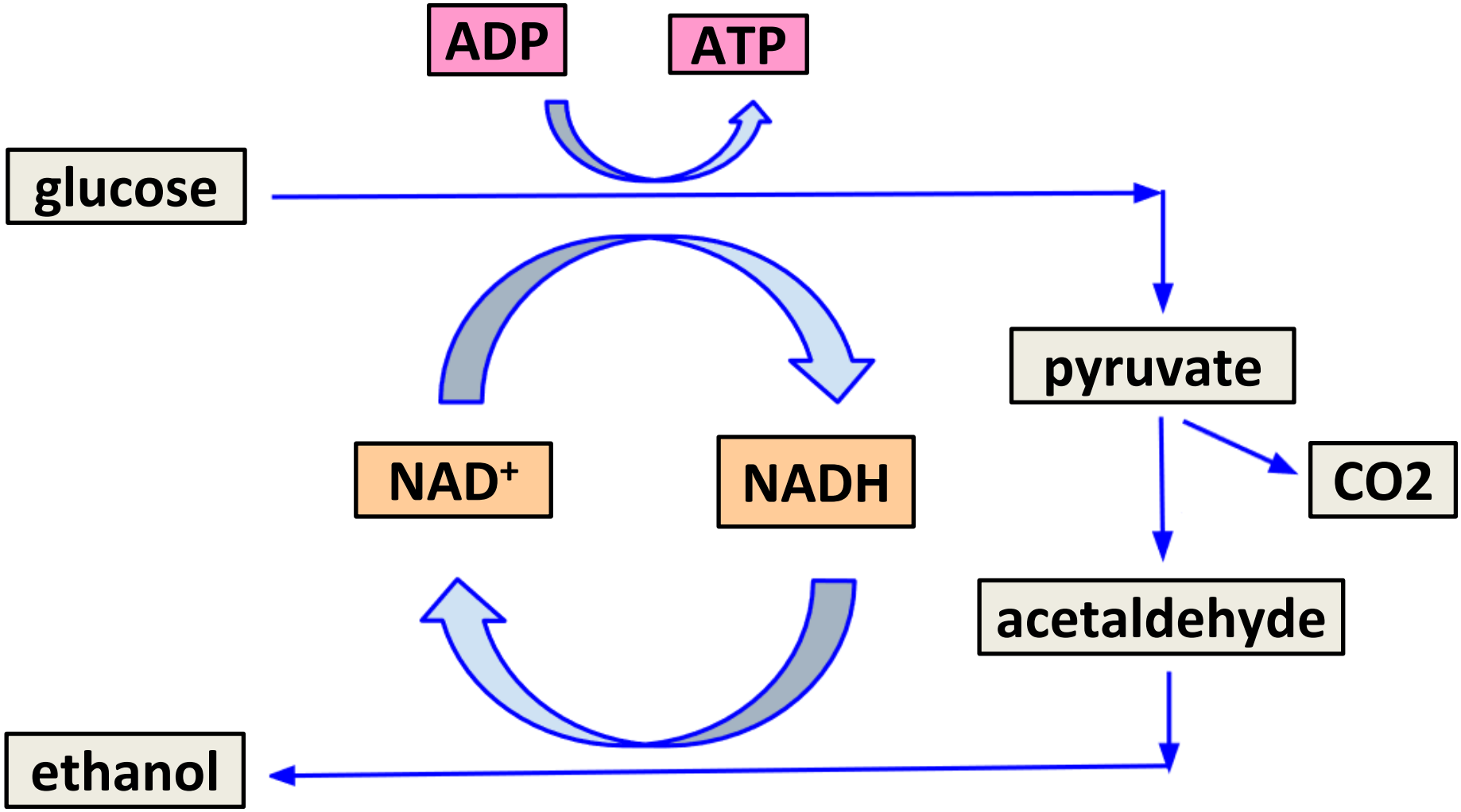
Acetaldehyde

Ethanol

Ethanol is just a waste product
→ excreted

Yeast must ensure that alc.deH has greater activity than pyr. decarb.

Glucose to ethanol: reaction summary



Part 2. Yeast biology, diversity, engineering

“YEAST” covers a wide range of wild, domesticated, and engineered organisms. Different jobs need different yeasts

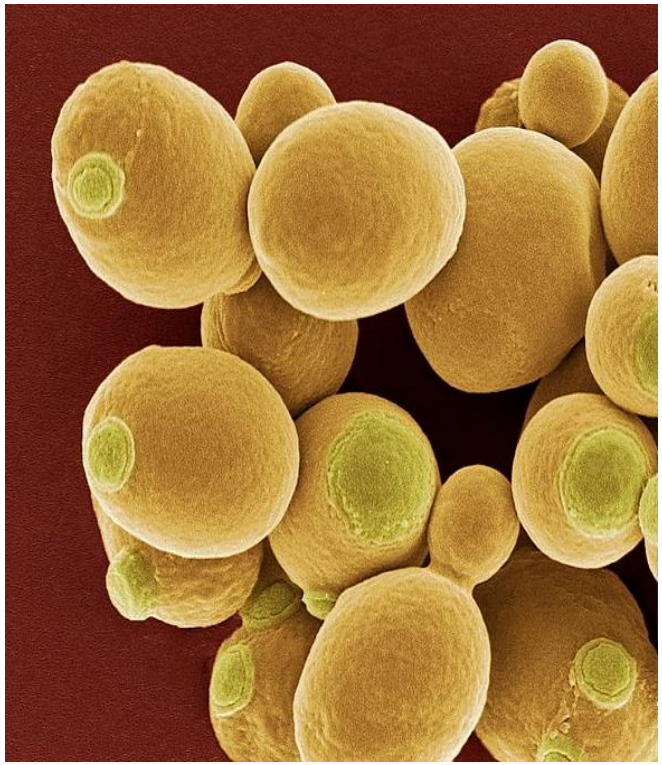
“ENGINEERING” here refers to both modifying the genome of the organism and modifying its environmental conditions

Optimising and controlling fermentation

- To optimise fermentation, we need to control:
  - BIOLOGY
    - Choice of organism
    - Wild-type vs. mutant vs. GMO
  - MEDIUM
    - Choice of nutrients
    - Special additions?
  - ENVIRONMENT
    - Aerobic / anaerobic
    - Temperature
  - TIMING
    - Stage of growth

Environment, medium, timing

- Need to match conditions to organism needs
- Need to optimise conditions to process needs



Saccharomyces yeast

Temperature: 30 C

pH 5 (mildly acidic)

Initially aerobic (rapid growth)

Organism needs

Switch to anaerobic for alcoholic fermentation

Process needs

Environment, medium, timing

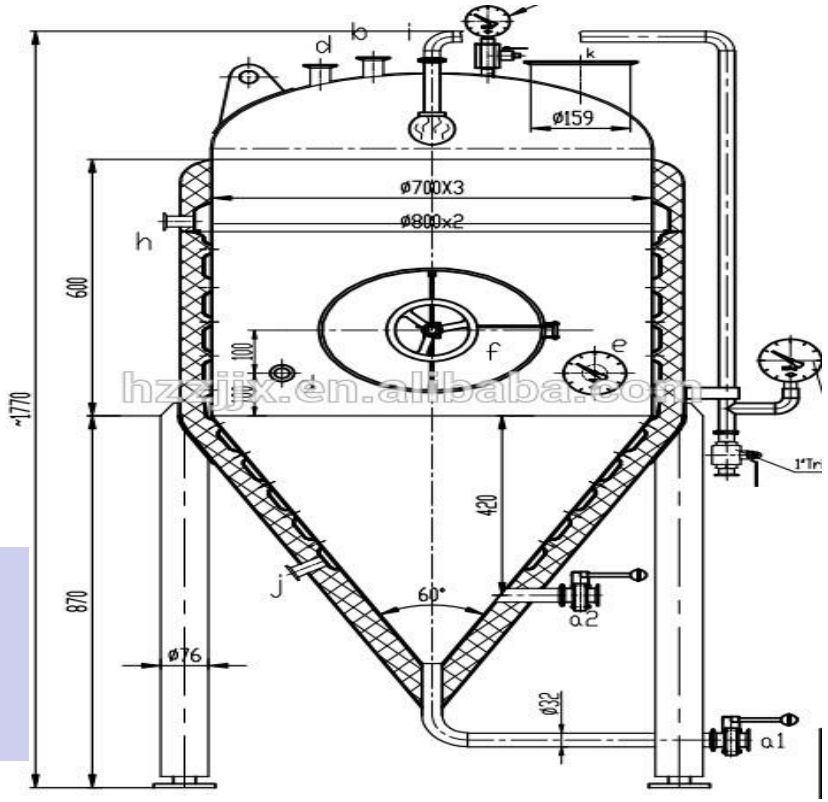
The aerobic / anaerobic switch

- *Saccharomyces* is a facultative anaerobe; can grow both aerobically (respiration) and anaerobically (fermentation)
- Aerobic growth is faster, but produces little ethanol

… so …

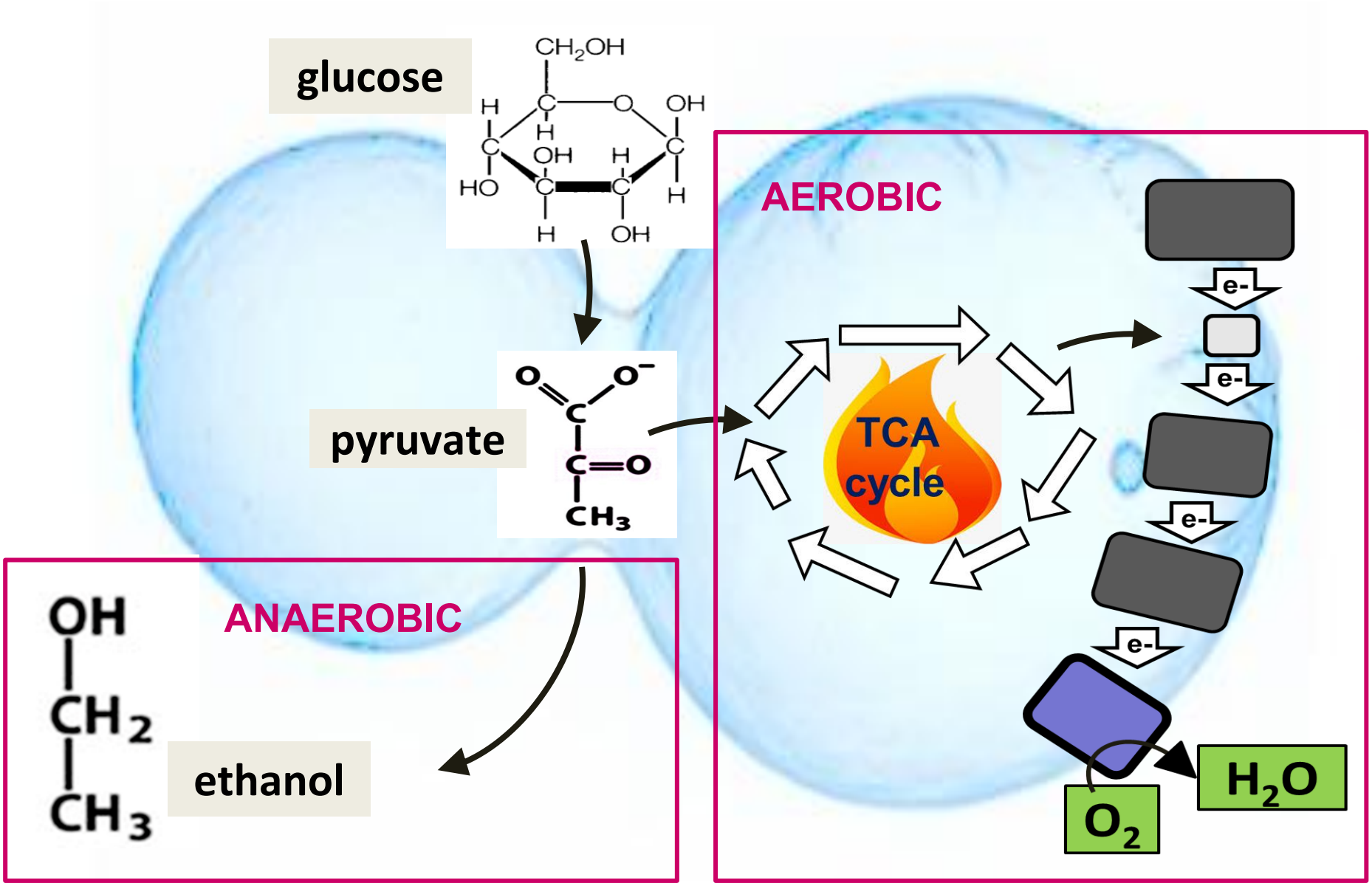
- Grow aerobically to build up biomass, then switch to fermentation

Need engineering AND biology for effective large-scale fermentation



Environment, medium, timing

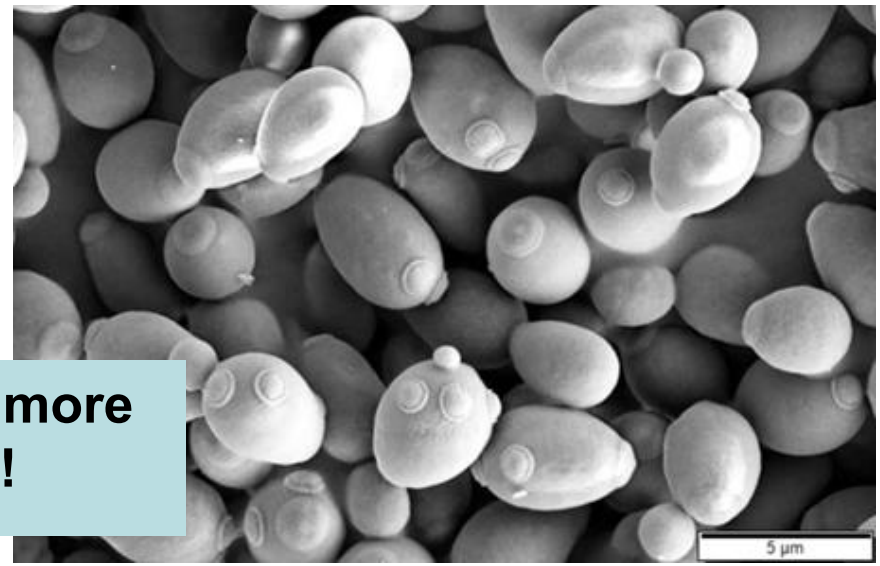
The aerobic / anaerobic switch



Biology: *Saccharomyces cerevisiae*

- **Eukaryotic, heterotrophic, facultative anaerobic**
- **Unicellular, divides by budding**
- **Complex genome – 16 chromosomes, 12 Mb**

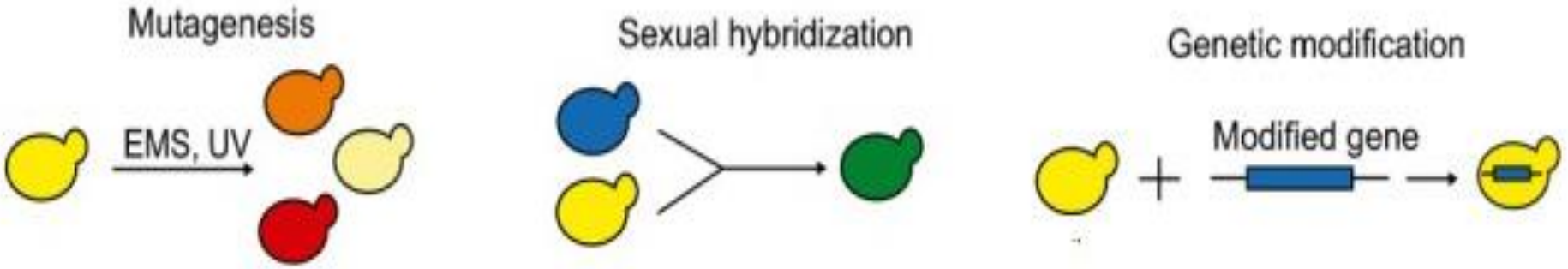
  (compare to E.coli: 1 chromosome, 4 Mb)
- **Generally Recognised As Safe (GRAS)**
- **Useful model organism**
- **Useful as host for heterologous expression**



YEAST means so much more than just making booze !

Biology: Diversity of yeast strains

Natural diversity



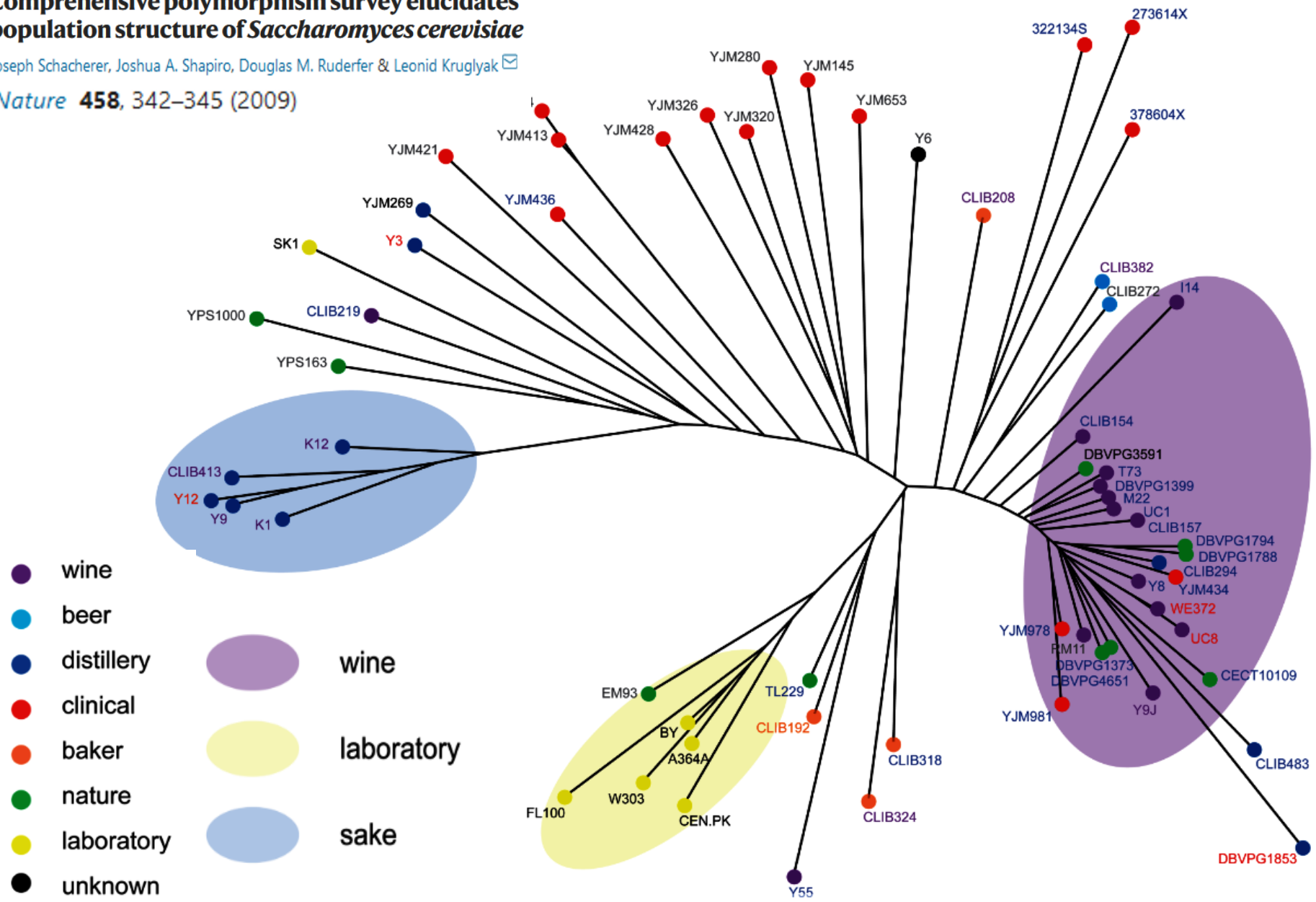
Artificial diversity

What does “natural” diversity mean for yeast?

Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*

Joseph Schacherer, Joshua A. Shapiro, Douglas M. Ruderfer & Leonid Kruglyak

Nature **458**, 342–345 (2009)



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“The majority of wine yeasts are members of a single subpopulation…this is strong evidence of a single domestication event of yeast for winemaking, followed by human-associated migration of wine yeast all over the world”

“The second major group contains strains used for sake production, providing evidence for a second independent domestication event”

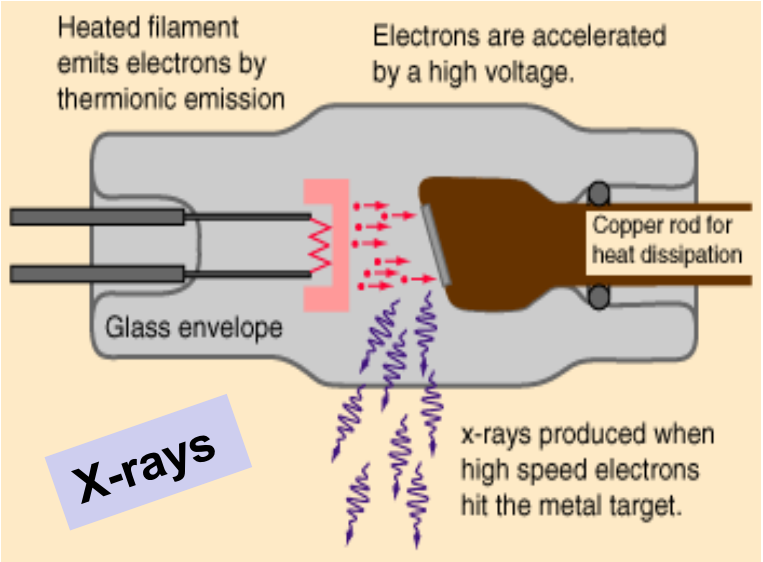
→ Yeast genomes constantly change, depending on what desirable phenotypes humans are selecting for

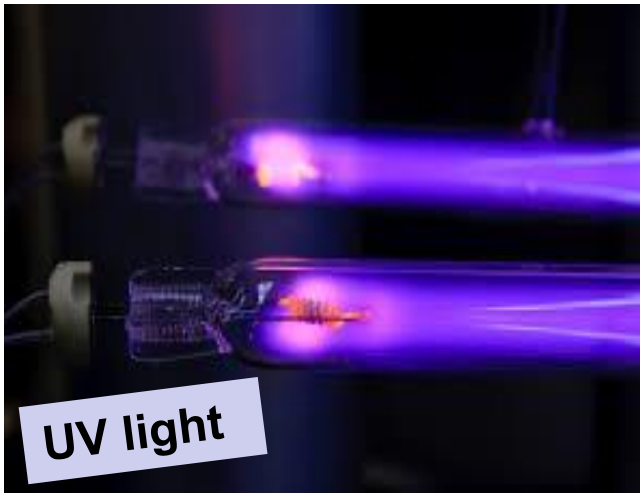
→ Humans help to generate diversity (is this ‘natural diversity’?)

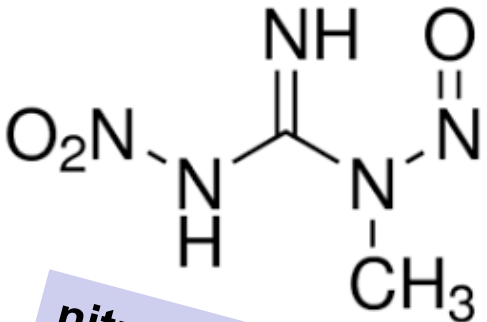
→ Humans and yeasts are CO-EVOLVING

Diversity can be generated by mutagenesis…

- **Desirable traits generated by mutation + selection :**
  - **High ethanol yield**
  - **Ethanol tolerance**
  - **Thermotolerance**
  - **Better sugar utilisation**



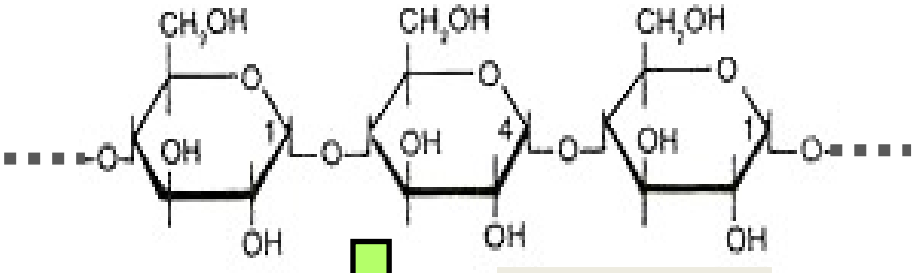




nitroso-guanidine

Radiation and chemicals are very useful for strain improvement by mutation

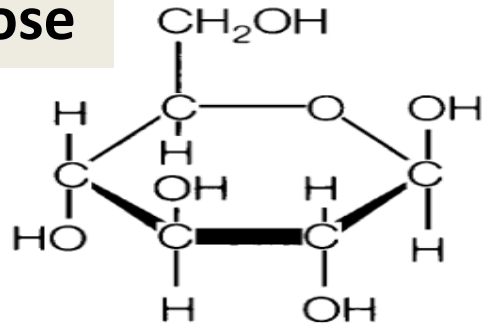
…or modifications can be via genetic engineering

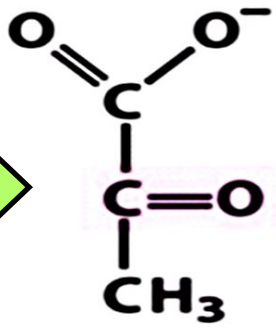


starch

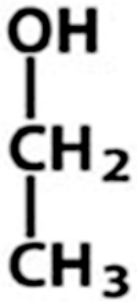
- Delete genes for glycerol synthesis
- Add starch metabolic genes
- Add xylose metabolic genes

glucose



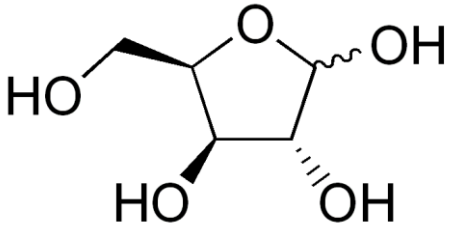


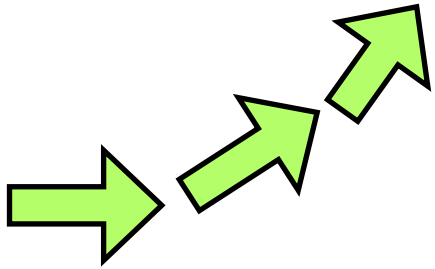
pyruvate



ethanol

xylose





The future: Yeast 2.0 project

- **An ambitious international synthetic biology experiment to rebuild the entire yeast genome … but WHY ?**

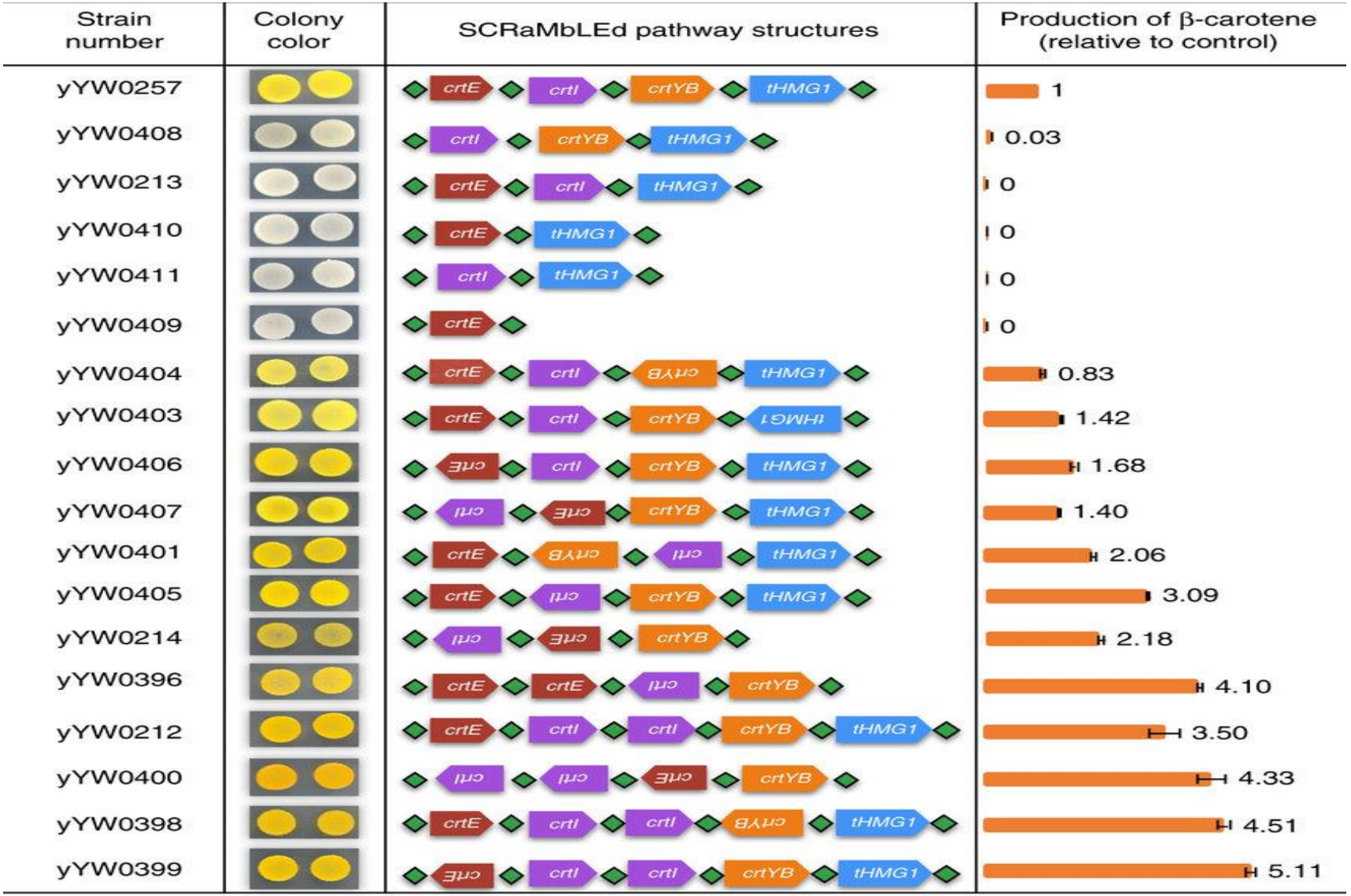
1. To remove all introns and other 'junk DNA'
→ streamlined genome → faster, more reliable growth (?)

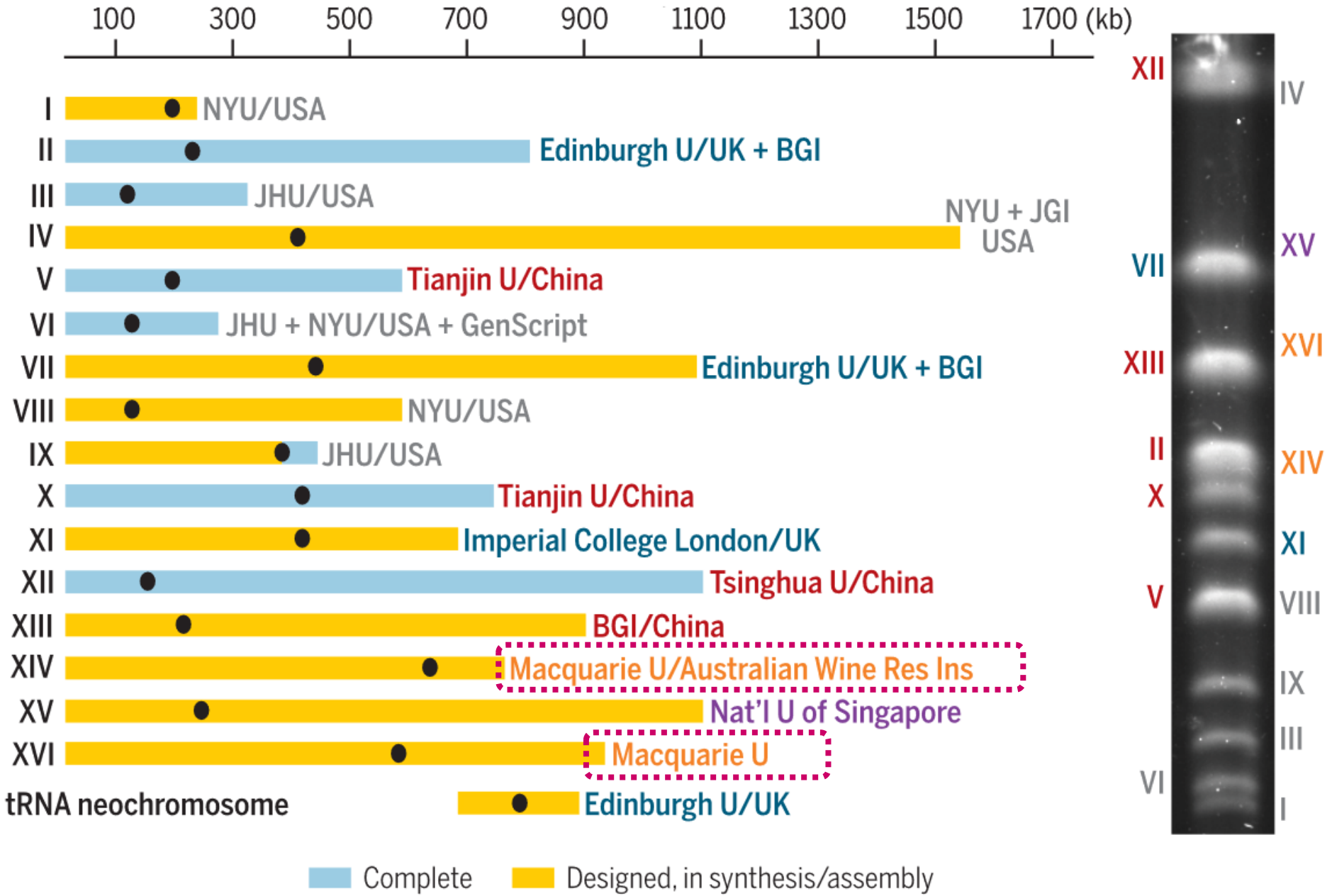
2. Add SCRaMbLE functionality – loxP sites put between genes to enable Cre-mediated DNA shuffling of genome

The future: Yeast 2.0 project

SCRAMBLE: Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution

NATURE COMMUNICATIONS (2018) 9:1935



The future: Yeast 2.0 project

Richardson et al., Science 355, 1040 –1044 (2017)