Connecting Growth Control and Stress Response in Yeast

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System-Level Diagram for Regulation in Bacteriophage λ

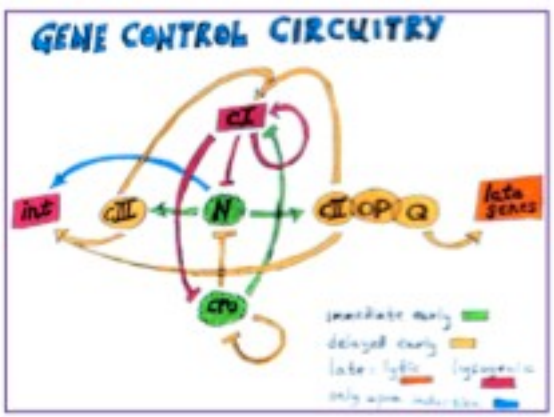
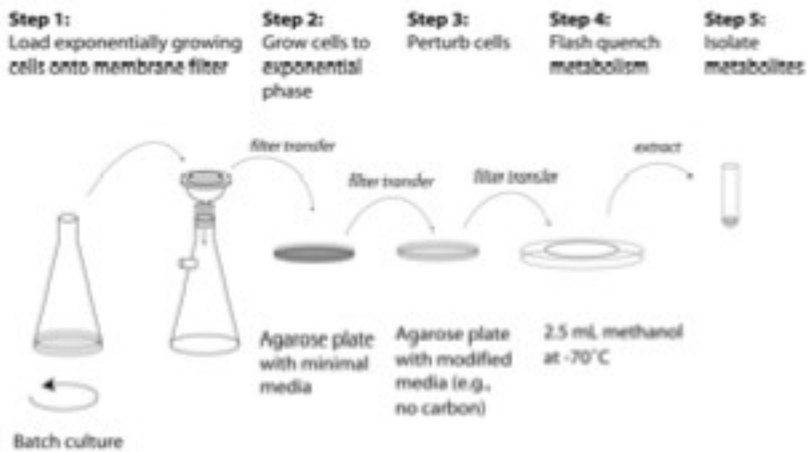


Diagram by Ira Herskowitz, ca. 1975

The Metabolic Map

The regulation of the network of bio-chemical reactions that underlies cellular growth and function presents a nearly ideal test bed for the ideas and methods of "system-level" biology because most of the biochemistry is already reasonably well understood.

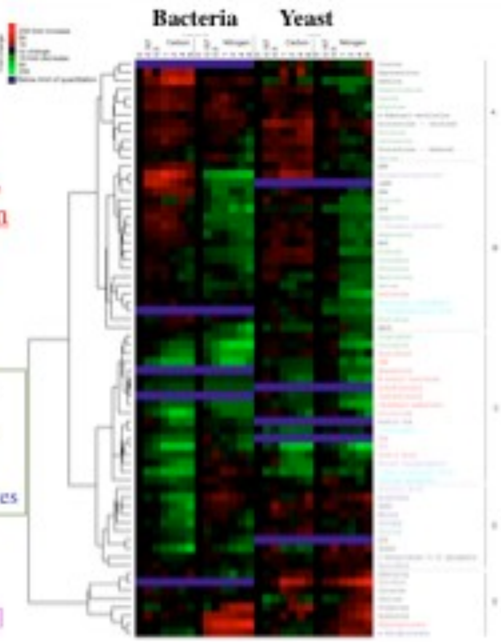
A Simple Technique for Fast Perturbation and Sampling of Exponentially Growing Cultures



Metabolite abundances during carbon vs. nitrogen starvation of *E. coli* and *S. cerevisiae*

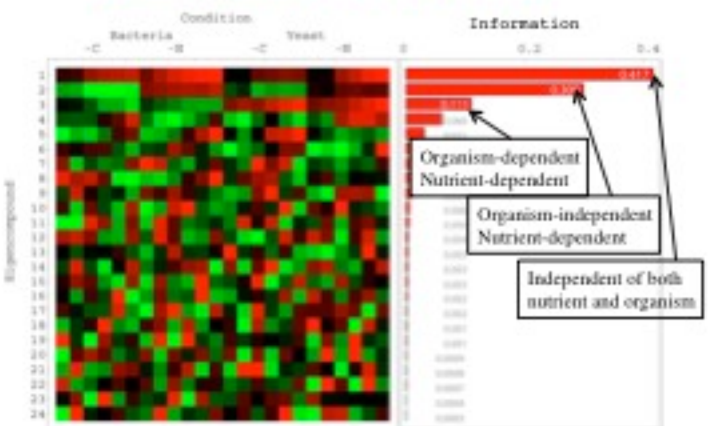
- Data clusters well
- Responses similar across organisms
- Many > 10X changes

[Brauer et al., 2006]

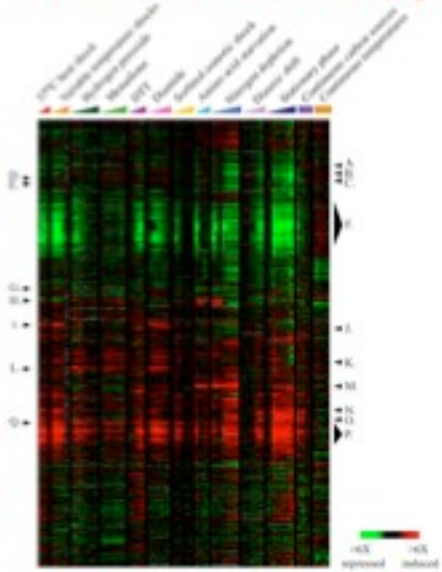


Singular Value Decomposition Analysis

Identifying Metabolite- and Organism-Specific Metabolomic Responses to Starvation



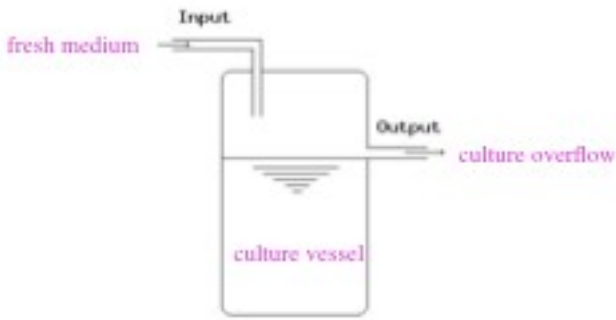
"Environmental Stress Response"



[Gasch et al., 2000]

Jacques Monod and Leo Szilard at Cold Spring Harbor

The Chemostat: Continuous Culture at Steady State



Rate-limiting nutrient: altered concentration in the fresh medium input results in a change in density in the culture vessel.

Theory of the Chemostat

- Combined relation predicts exponential growth or washout unless terms balance

$$\frac{1}{n}\frac{dn}{dt} = \mu - D$$

n = cell number
μ = growth rate
D = dilution rate

- Steady state is reached when $\mu = D$
- D is determined by the experimenter

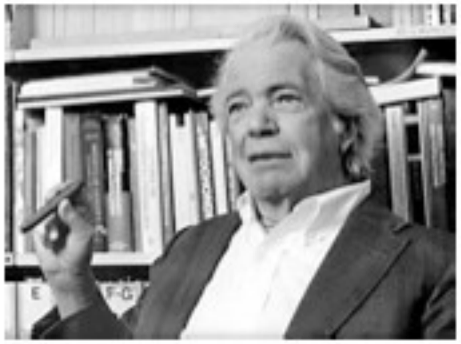
Growth rate accomodates to environment

Bacteria and yeast will reach steady state for any $D < \mu_{max}$ under virtually any kind of nutrient limitation regime, where μ_{max} is the maximum growth rate in that regime.

$$\mu = \mu_{\max} \frac{S}{S + K_S}$$

[S is the limiting nutrient concentration at the steady state imposed by setting the dilution rate D, and hence μ]

The Concept of "Balanced Growth"



Ole Maaløe

Growth rate should be proportional to the number of ribosomes in balanced growth.

Dilution Rate Series

Experimental design: Run chemostats with a variety of limiting nutrients (glucose, phosphate, sulfate, ammonia and (in suitable auxotrophs) uracil or leucine at a number of different growth rates (μ) by setting the dilution rate (D).

Measure:

1. **Bud Index** [fraction of unbudded cells (i.e. in G0/G1)] and DNA content (FACS).

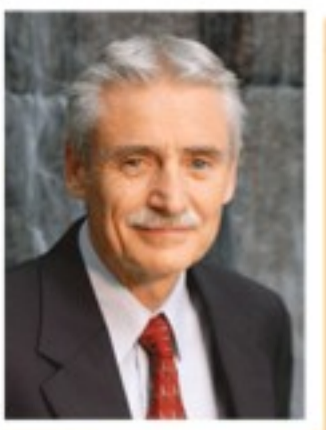
2. **Nutritional parameters** (residual glucose, ethanol, etc.)

3. **Gene expression** (DNA microarrays)

[Brauer et al., 2008]

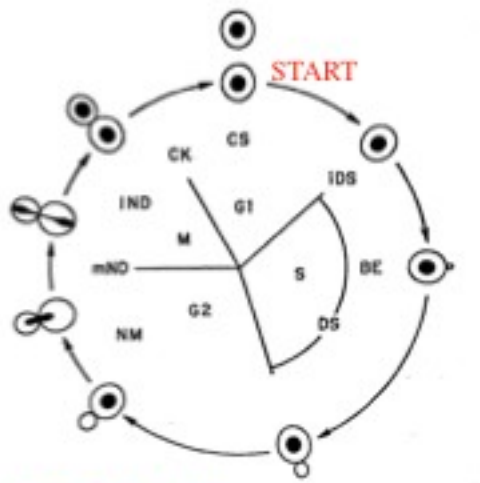
Genetic Analysis of the Yeast Cell Division Cycle

The success of phage geneticists in learning about the life cycle, gene regulation and morphogenesis of bacterial viruses inspired Hartwell to collect conditional-lethal (temperature-sensitive) mutants of yeast with which he was able to identify a subset that are specifically defective in the progress of the cell division cycle.



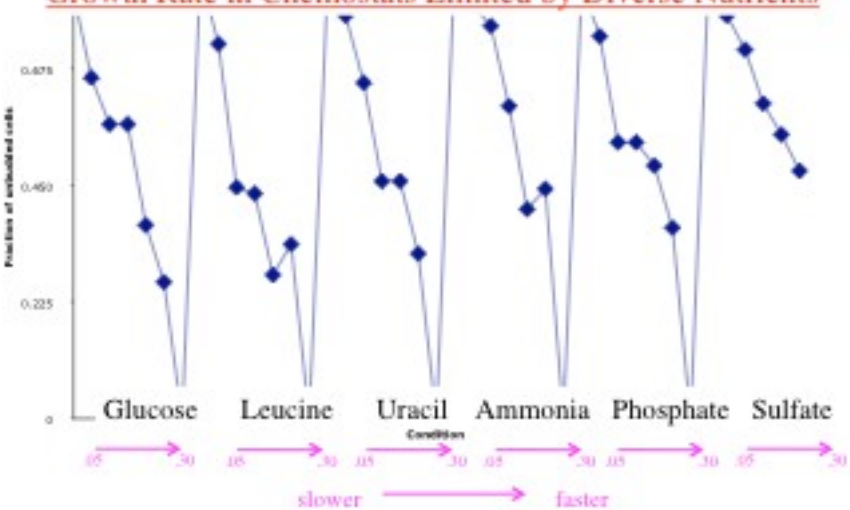
Lee Hartwell

Landmarks of the Yeast Cell Division Cycle



[Hartwell et al., 1974]

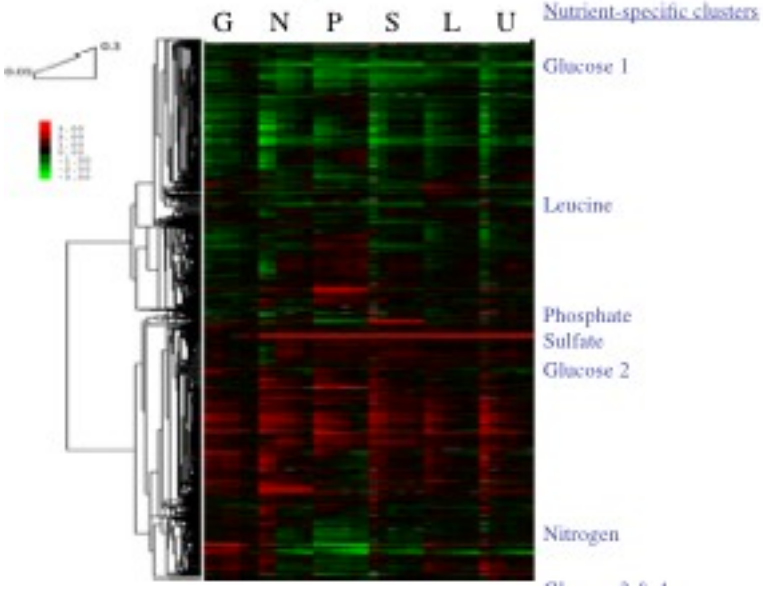
Fraction of Unbudded Cells as a Function of Growth Rate in Chemostats Limited by Diverse Nutrients



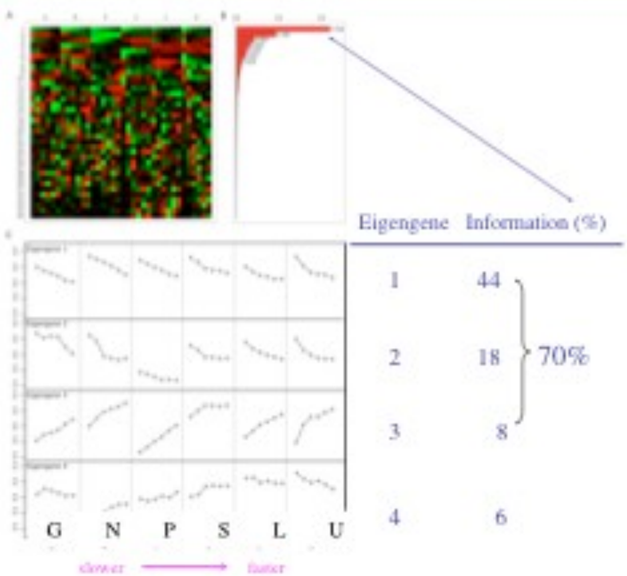
[Brauer et al., 2008]

Growth rate (μ) = Dilution rate (D)

Hierarchical Clustering of Dilution-Rate Series Expression Data



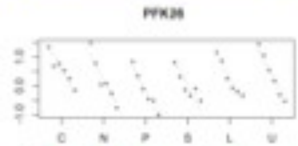
Several Orthogonal Eigengenes are Correlated with Growth Rate



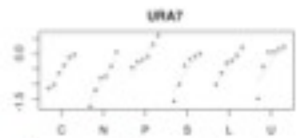
Genes ranked by response to growth rate

Three classes of genes

- strong correlation, all limiting nutrients, negative slope
- strong correlation, all limiting nutrients, positive slope
- growth rate independent, good fit to zero slope

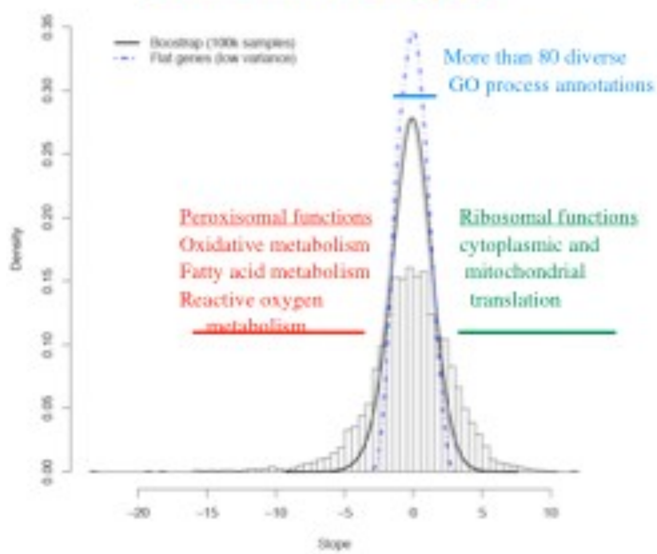


Top of list: negative slope

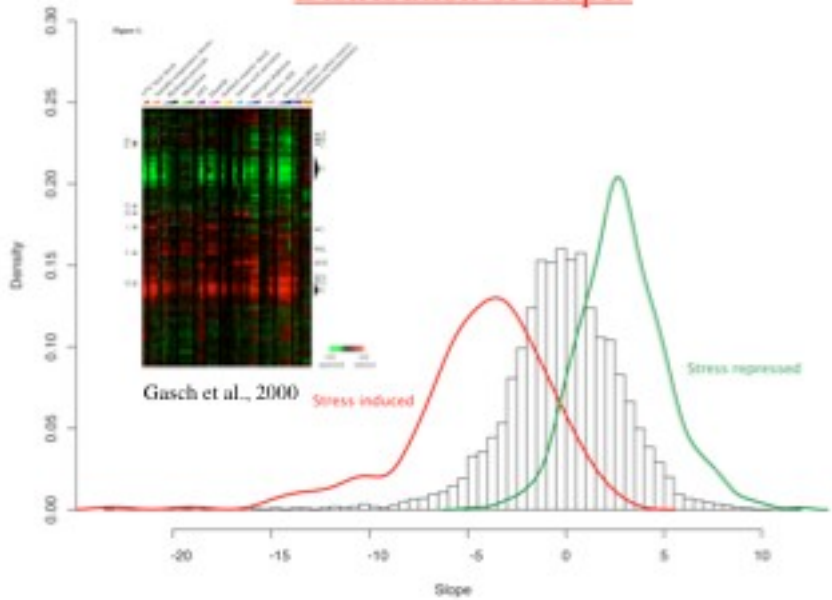


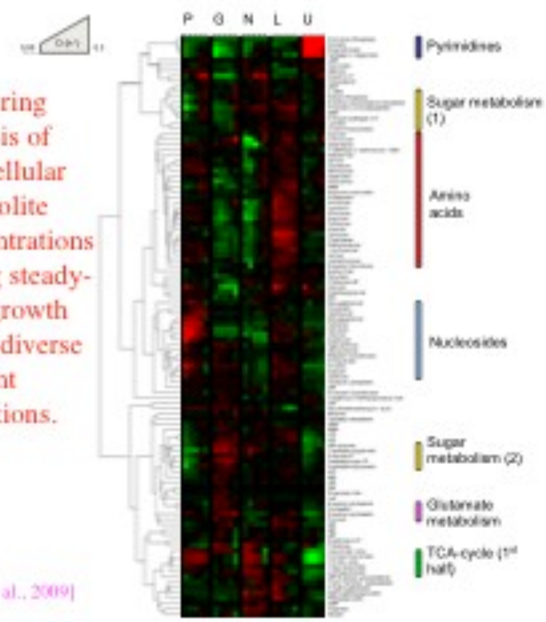
Bottom of list: positive slope

Distribution of Slopes



Distribution of Slopes





Clustering analysis of intracellular metabolite concentrations during steady-state growth under diverse nutrient limitations.

Boer et al., 2009]

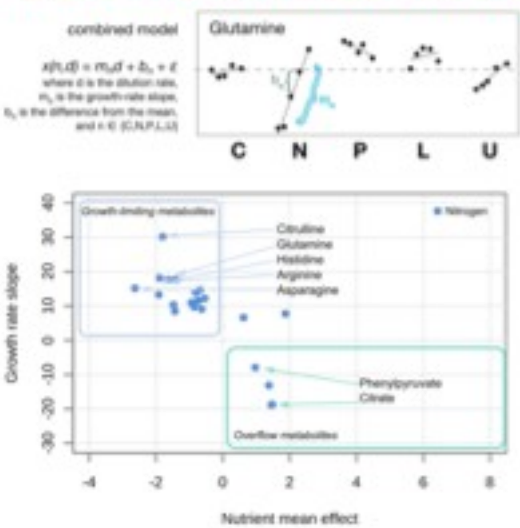
Combined Data Model

$$x(n,d) = m_n d + b_n + \varepsilon$$

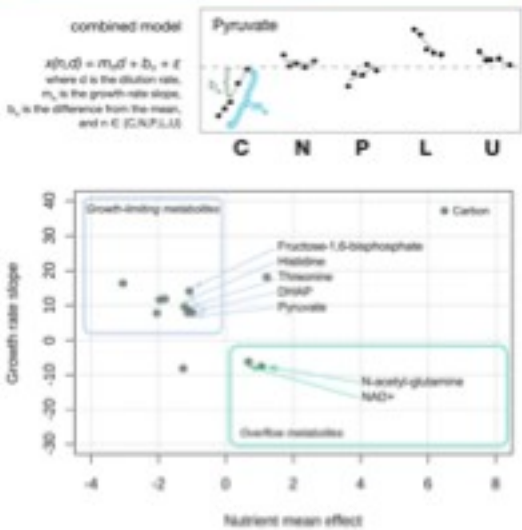
d is the dilution rate,
m_n is the growth rate slope,
b_n is the difference from the mean level of all cultures (nutrient mean effect).

[Patrick Bradley; Boer et al., 2009]

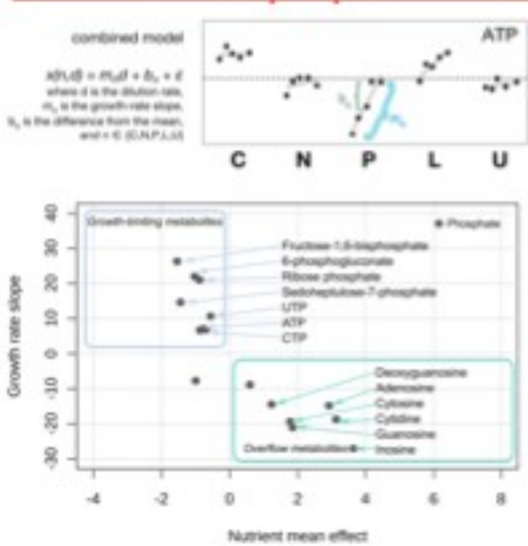
Defining growth-limiting intracellular metabolites when extracellular ammonium is limiting



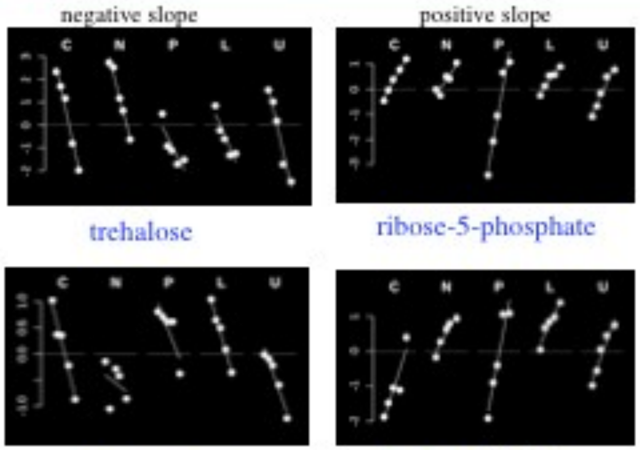
Defining growth-limiting intracellular metabolites when extracellular glucose is limiting



Defining growth-limiting intracellular metabolites when extracellular phosphate is limiting



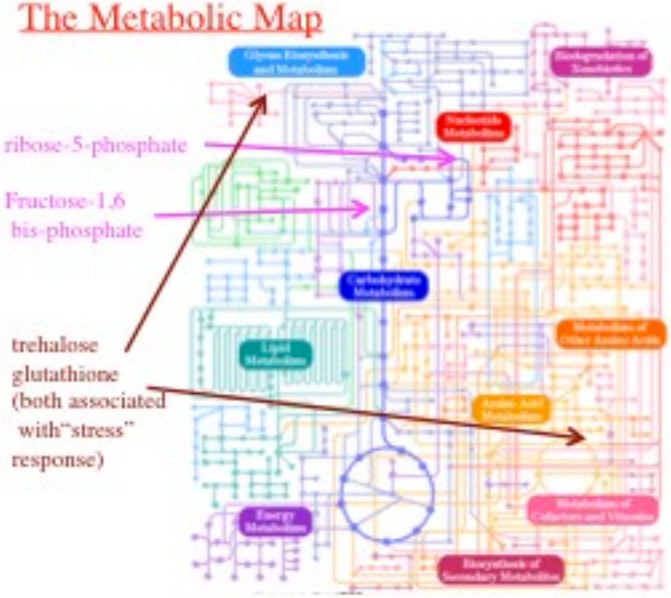
Few intracellular metabolites are correlated with growth rate, independent of the nature of the external nutrient limitation



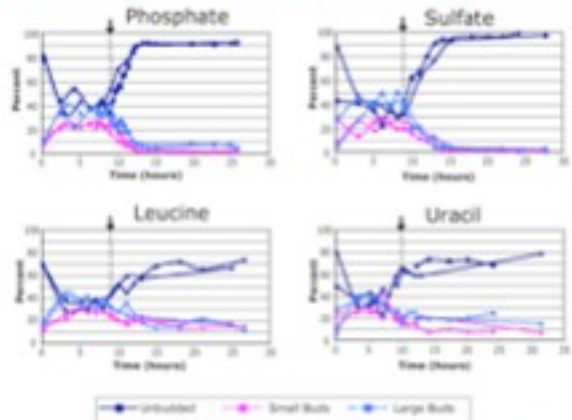
trehalose — ribose-5-phosphate

glutathione — fructose-1,6-bis phosphate

The Metabolic Map



Cell Cycle Arrest in Diverse Starvation Regimes

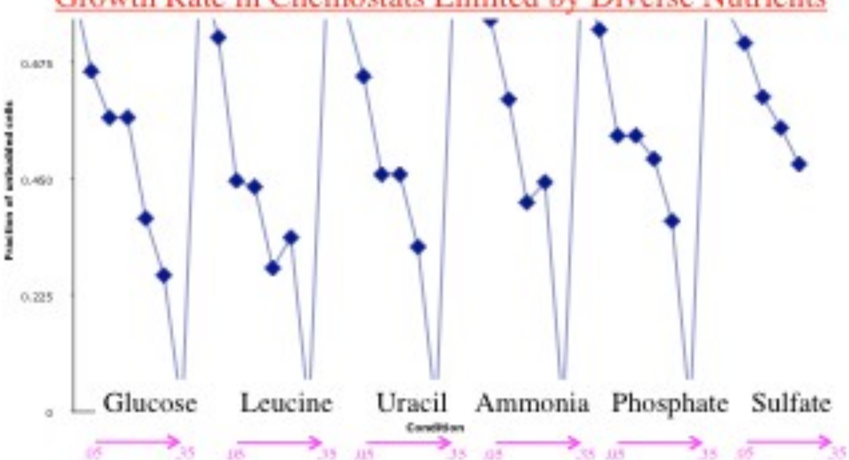


"natural" limitations ----> evolved, organized response including **cell cycle arrest**, absent when auxotrophs starve.

[Saldanha et al., 2004]

Fraction of cells in G1 decreases with increasing growth rate

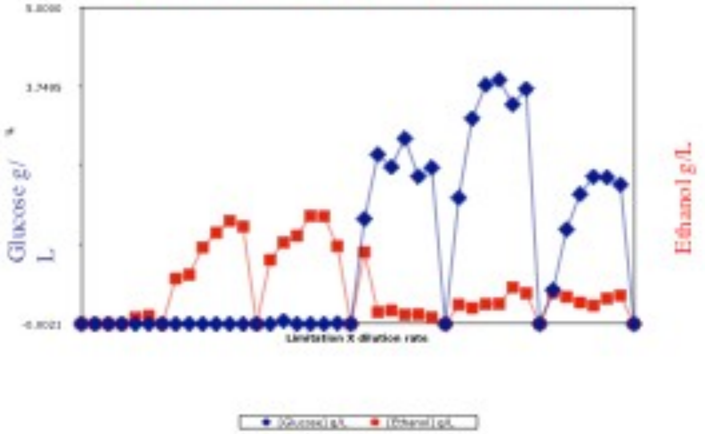
Fraction of Unbudded Cells as a Function of Growth Rate in Chemostats Limited by Diverse Nutrients



[Brauer et al., 2008]

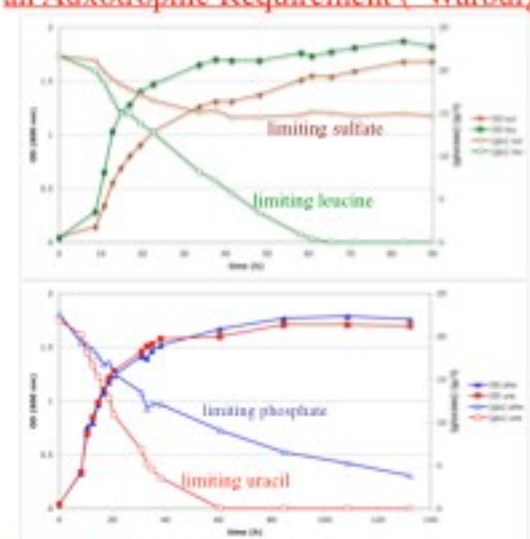
Growth rate (μ) = Dilution rate (D)

Residual Glucose and Ethanol Concentrations



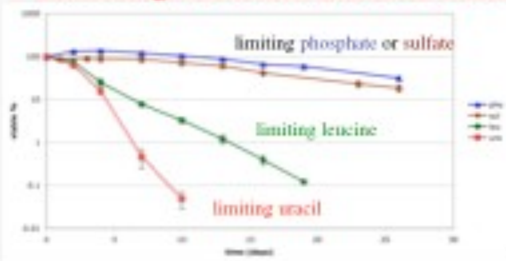
In 1931, Otto Warburg first called attention to the apparently uncontrolled fermentation in cancer cells and tissue slices

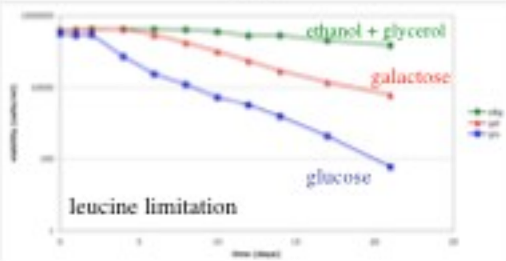
Excess Glucose is Consumed when Growth is Limited by an Auxotrophic Requirement ("Warburg Effect")



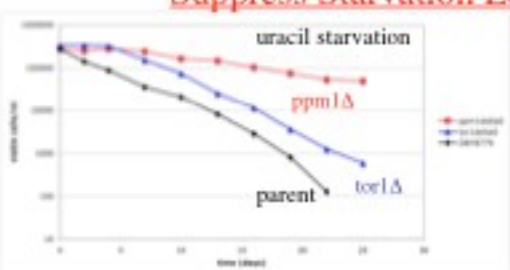
[Boer et al., 2008]

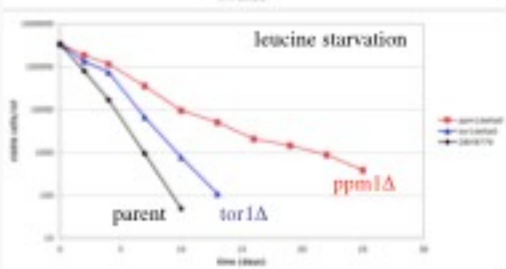
Survival During Starvation Depends on the Limiting Nutrient and the Carbon Source





*ppm1*Δ and *tor1*Δ Mutants Suppress Starvation Lethality

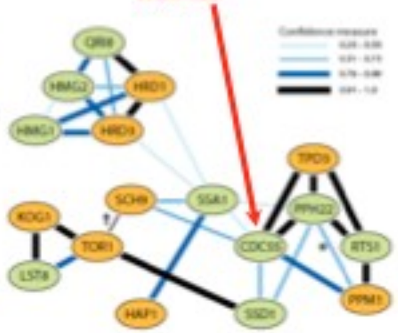




Genes Implicated by Survival Screen are Functionally Related

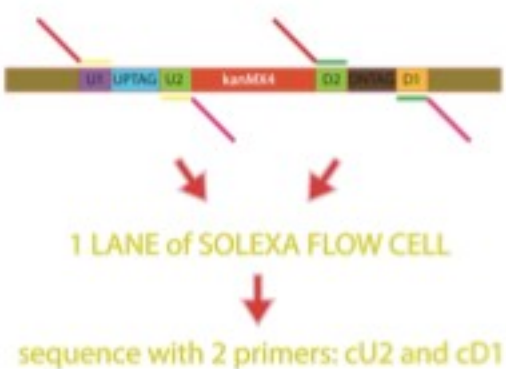
Analysis in Biopixie

The mutations recovered are in genes concentrated in a functional network that is implicated in many diverse processes. Orthologs of many of these same genes are proven proto-oncogenes or otherwise implicated in human cancer.

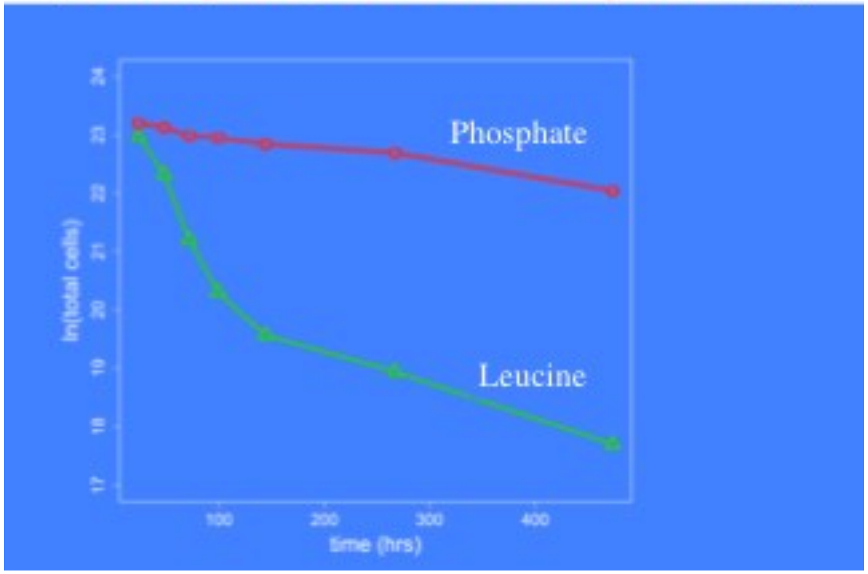


[Biopixie: Myers et al., 2005; Troyanskaya laboratory]

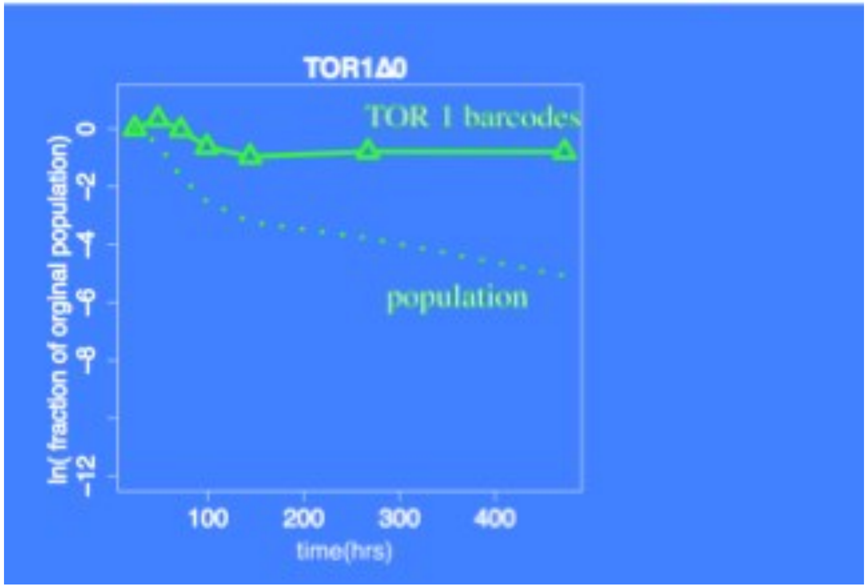
Deep sequencing of molecular barcodes



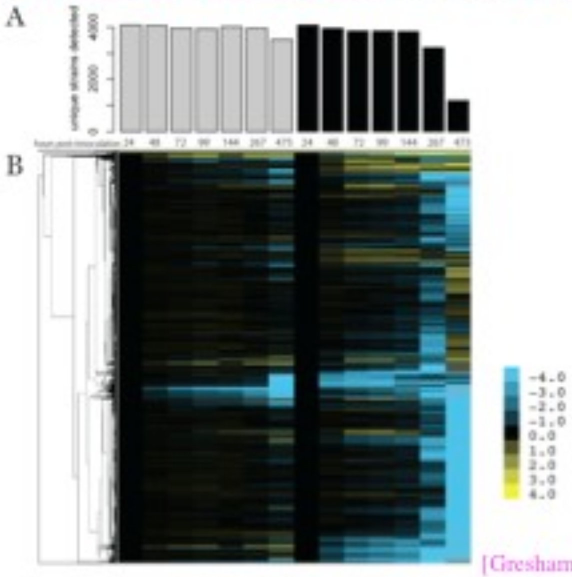
Total Population Survival during Starvation



Survival of TOR1Δ0 leucine starved

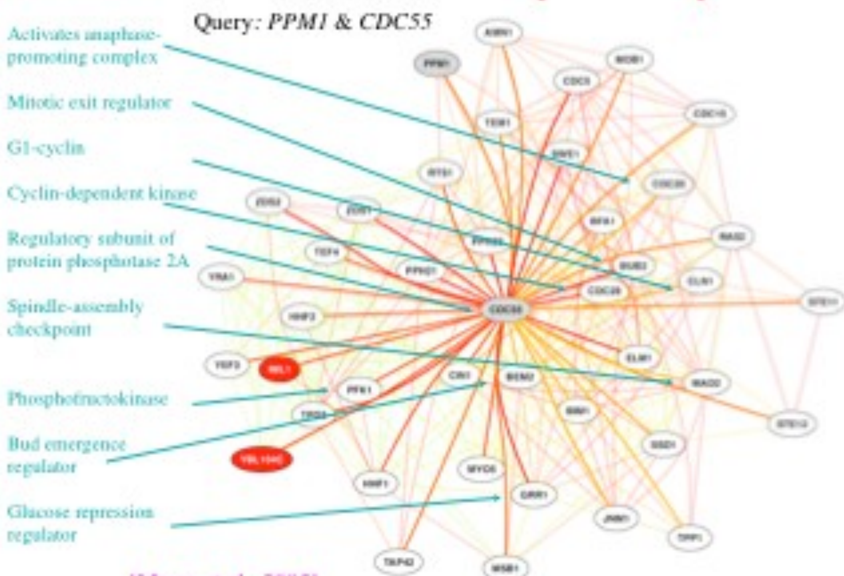


Clustering Genes by Relative Fitness During Starvation



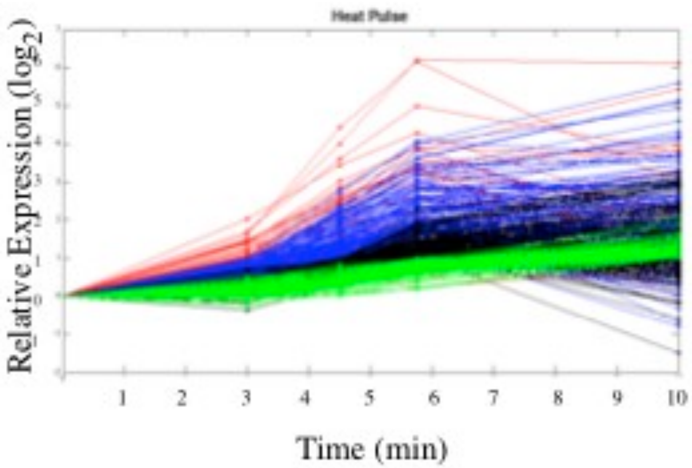
[Gresham et al., 2011]

Functional Network Relationships from Biopixie

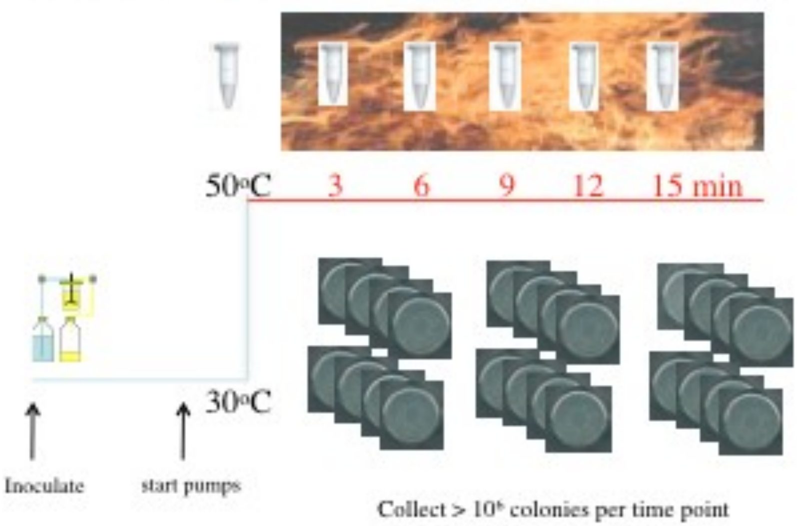


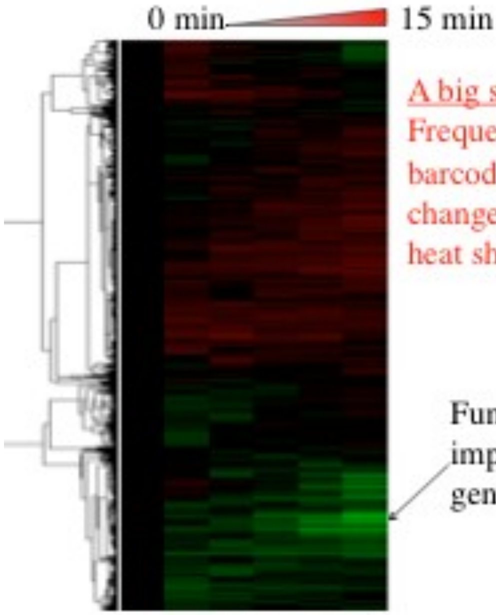
[Myers et al., 2005]

Genes Respond to Heat Shock at Different Rates



Heat Shock Fitness Profile Experimental Design



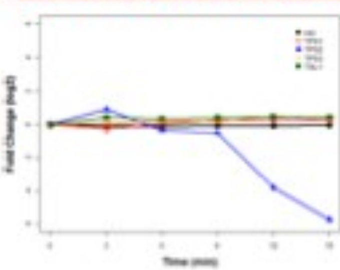


A big surprise:
Frequency of most barcodes does not change much during heat shock)

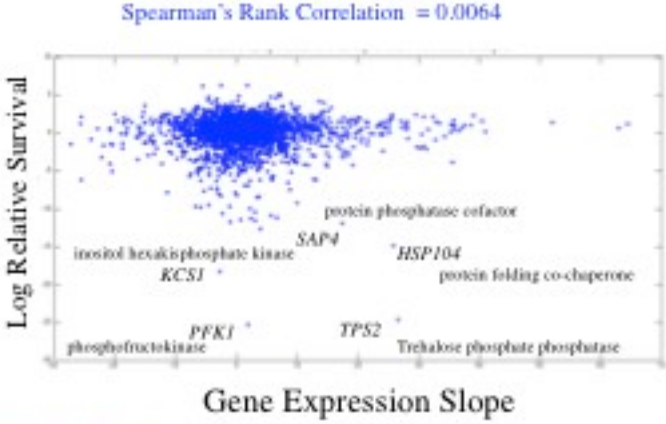
Functionally important genes

Annotated "Heat Shock Genes"

Trehalose Metabolism Genes



No Correlation between Gene Expression Change and Mutant Survival Response to Heat Shock



[Charles Lu]

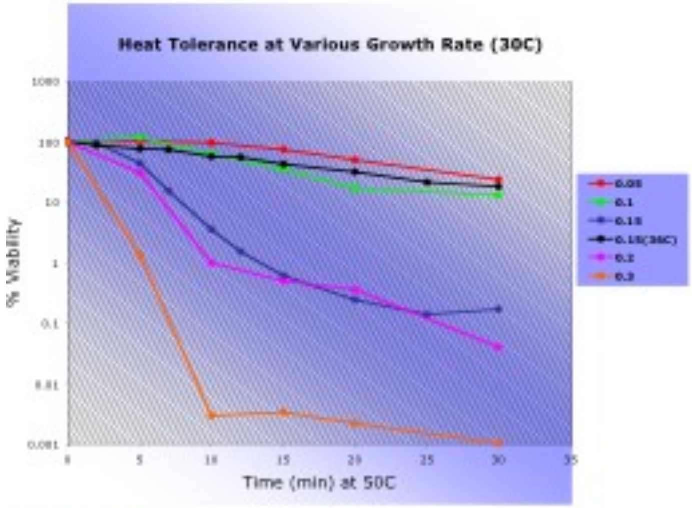
How Stressful is Slow Growth?

The literature assumes that the default growth pathway selected for by evolution is fast growth in rich medium on glucose.

An alternative view- slow growth rates under changing conditions may be a more important evolutionary adaptation.

A combination of slow growth in variable environments punctuated by bursts of fast growth in highly favorable glucose media may best describe the forces that selected the yeast that we see today.

Slower Growing Cells are more Stress Resistant



[Charles Lu]

Acknowledgements

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Olga Troyanskaya

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