

## Gene recruitment and adaptive reprogramming of gene regulation in yeast

Today the world is messages, codes and information.  
Tomorrow what analysis will break down our objects  
to reconstitute them in a new space?

What new Russian doll will emerge?

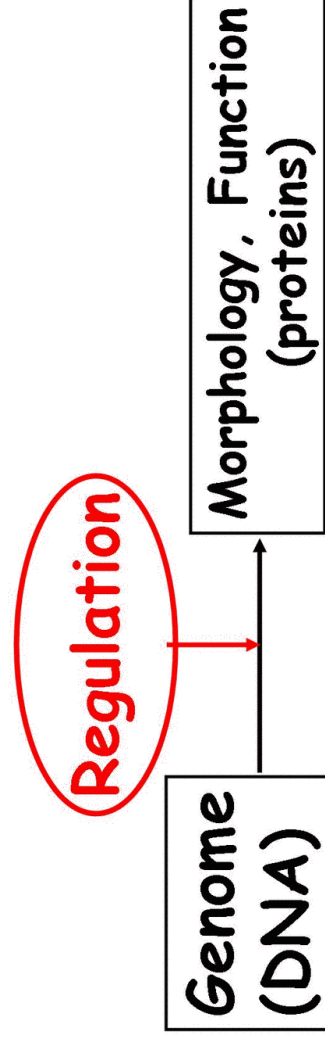
Francois Jacob, The Logic of Life  
(Originally published as: La Logique du Vivant: une histoire  
de l'heredite, 1970).

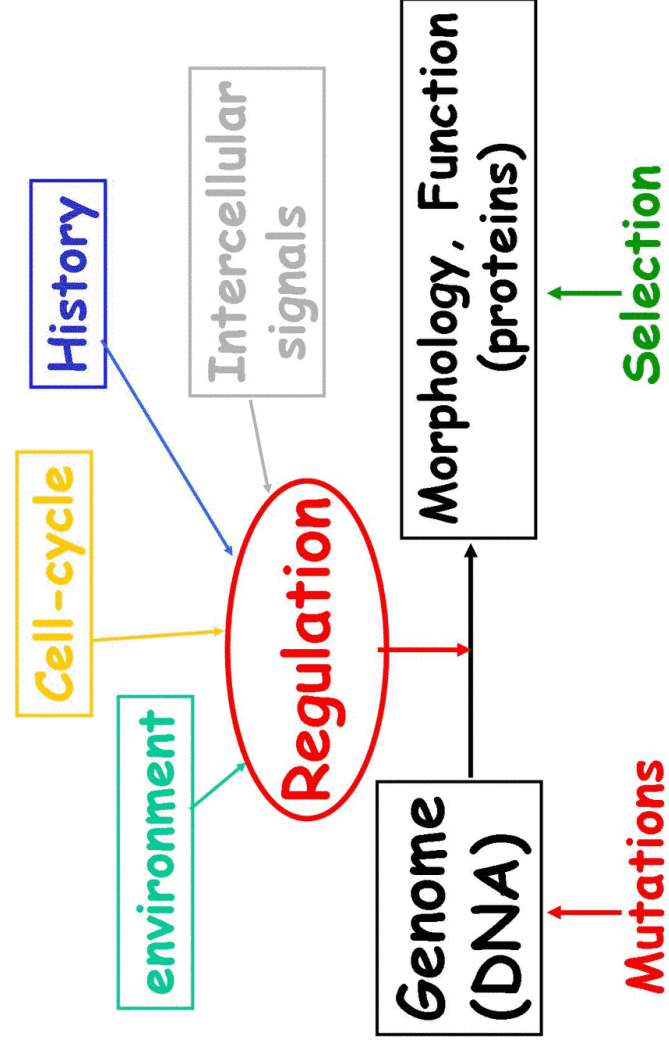
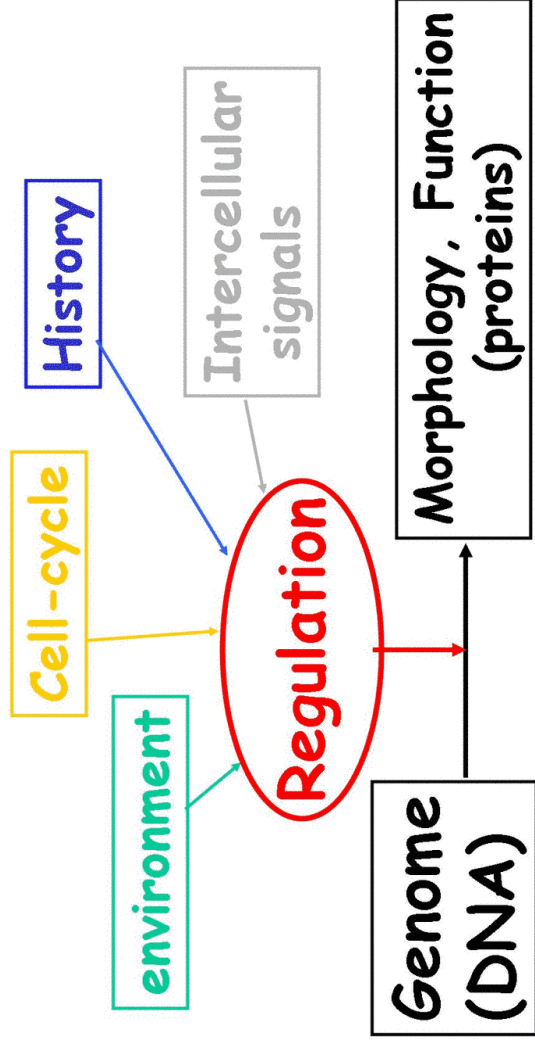
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Department of Physics  
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### Acknowledgments:

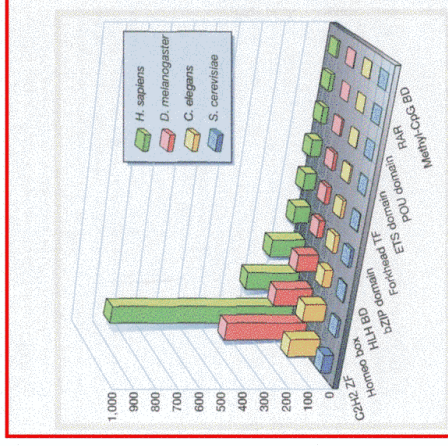
- Naama Brenner  
Dept. of Chemical Eng., Technion.
- Tali Dror
- Elad Stolovicki
- Shay Stern

- Genetic regulatory networks—an evolutionary perspective.
- The experimental setup; online measurements of the metabolic and regulatory dynamics in continuous cultures.
- Physiological adaptations of gene regulation; transient responses and steady-states.
- Gene-recruitment; population selection pressure, adaptive dynamics and long-term memory of an adapted cell state.





## Distribution of transcription factors and the emergence of cell types (cell states)



Nature 409, 832 2001.

~30,000 *Human* genes → **10<sup>9</sup>,000** possible different states  
 ~ 2000 transcriptional activators: ~**10<sup>600</sup>** states

but only ~250 cell types, constrained by regulatory proteins and their interactions → **the potential of genomes is far greater than that realized in evolution!**

## Summary of gene regulation main features :

- Span a huge combinatorial space.
- Network dynamics; highly interacting dynamical system spanning a wide range of time scales.
- Population effects are important; history, epigenetic inheritance.

**SCIENCE**

11 April 1975, Volume 188, Number 4184

*[Faded text from the original article]*

**We therefore propose that regulatory mutations account for the major biological differences between humans and chimpanzees.**

**Evolution at Two Levels in Humans and Chimpanzees**

Their macromolecules are so alike that regulatory mutations may account for their biological differences.

Mary-Claire King and A. C. Wilson

evidence concerning evolution at We suggest that in anatomy and way of life are more often based on changes in the genes of humans controlling the production of proteins. We therefore propose that regulatory mutations account for the major biological differences between humans and chimpanzees.

Similarity of Human and Chimpanzee Genes

**Regulatory evolution creates new combinations of gene expression and enables increase in the information content of genomes without expansion of gene number.**

Refs: Carroll, S. B., *PLoS Biology* 3: 1159-1166 (2005 );

Carroll, S.B. *Nature* 409, 1002 (2001);

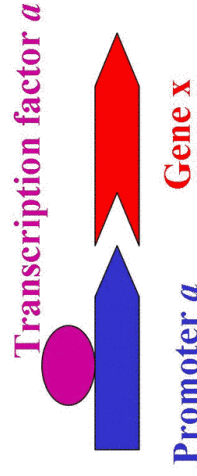
“The evolution of developmental pathways”; Wilkins 2002;

“From DNA to diversity”, Carroll, Grenier & Weatherbee 2001.

“Genomic regulatory systems, development and evolution”, Davidson, 2001 .

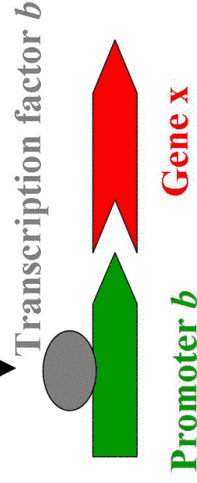
**Gene recruitment**

Gene **x** is active in specific cells and times according to promoter **a**.



**Gene recruitment**

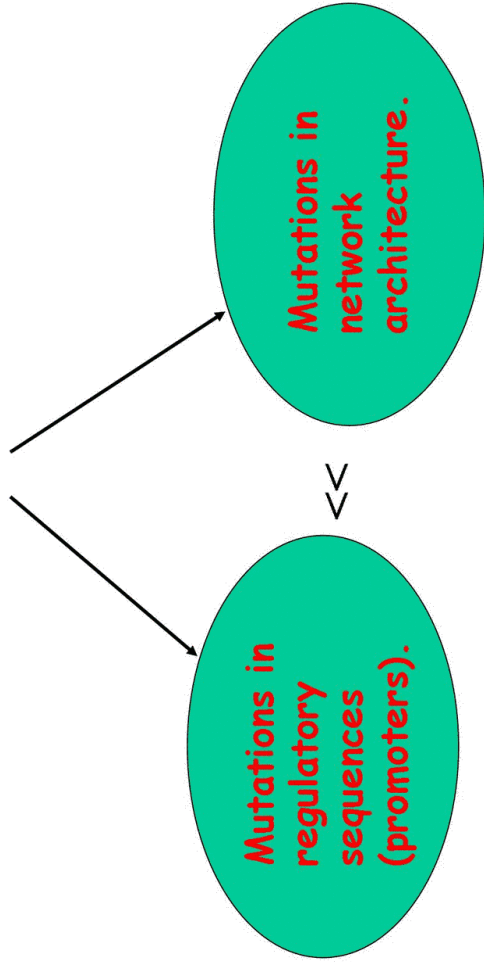
Gene **x** is recruited to a foreign regulatory system and active in different cells and times now according to promoter **b**.



**Gene recruitment can explain the evolutionary increase in the complexity of pathways and networks.**

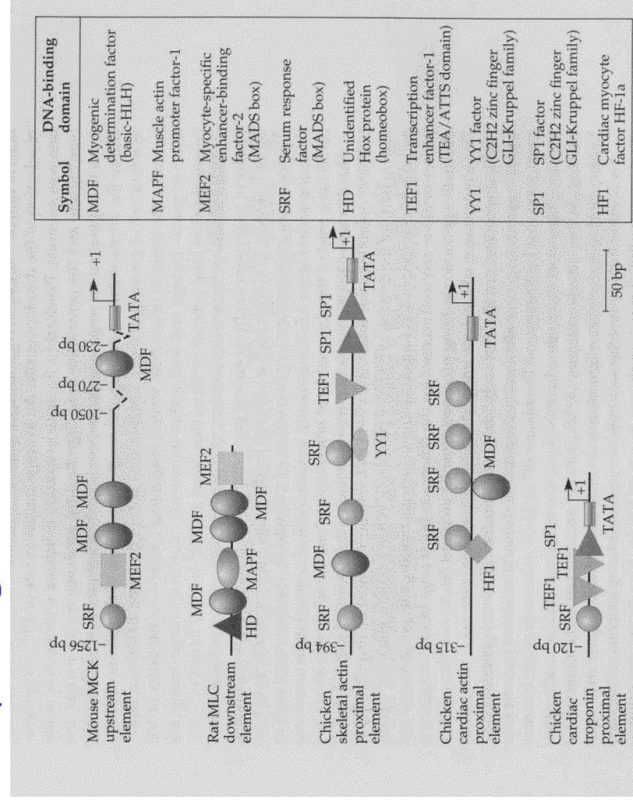
# Evolution of *cis*-regulatory systems

## Two Types of mutations



Effect of base pair substitutions in promoters is usually much weaker than mutations in network architecture.

## A "battery" of genes—combinatorics of TFs



ref: Arnone and Davidson 1997

## What type of evolutionary forces shaped genetic regulatory networks? and how?

Mimicking the natural evolutionary event of gene recruitment, we “rewire” the genome and measure the capacity of cells to adapt in different environments.

**Does cellular physiology support arbitrary gene recruitment events?**

**What is the evolvability potential of cell populations?**

Requires to open **time** and **population** dimensions.

Research in progress

## The chemostat

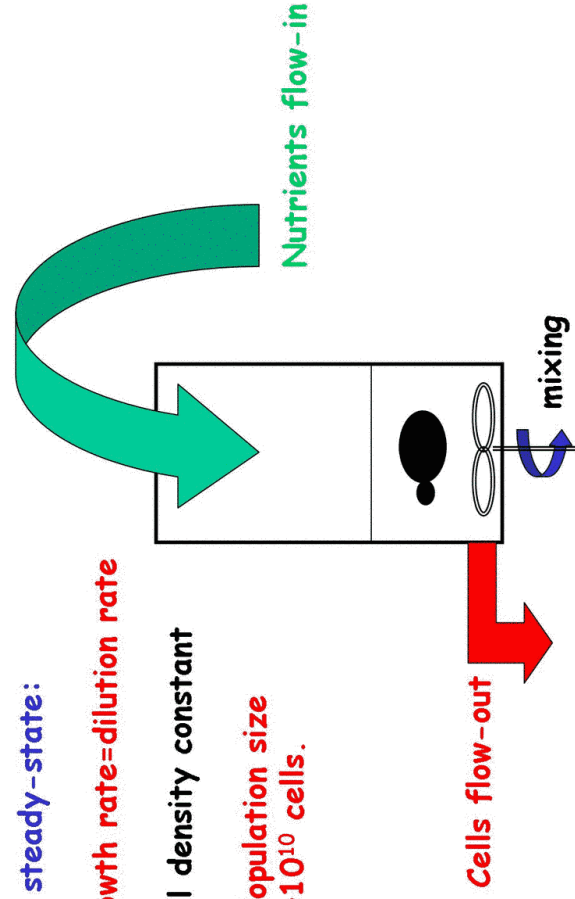
Novick and Szilard, PNAS 36, 708 (1950).

**At steady-state:**

**growth rate=dilution rate**

**Cell density constant**

**Population size  
~ $10^{10}$  cells.**

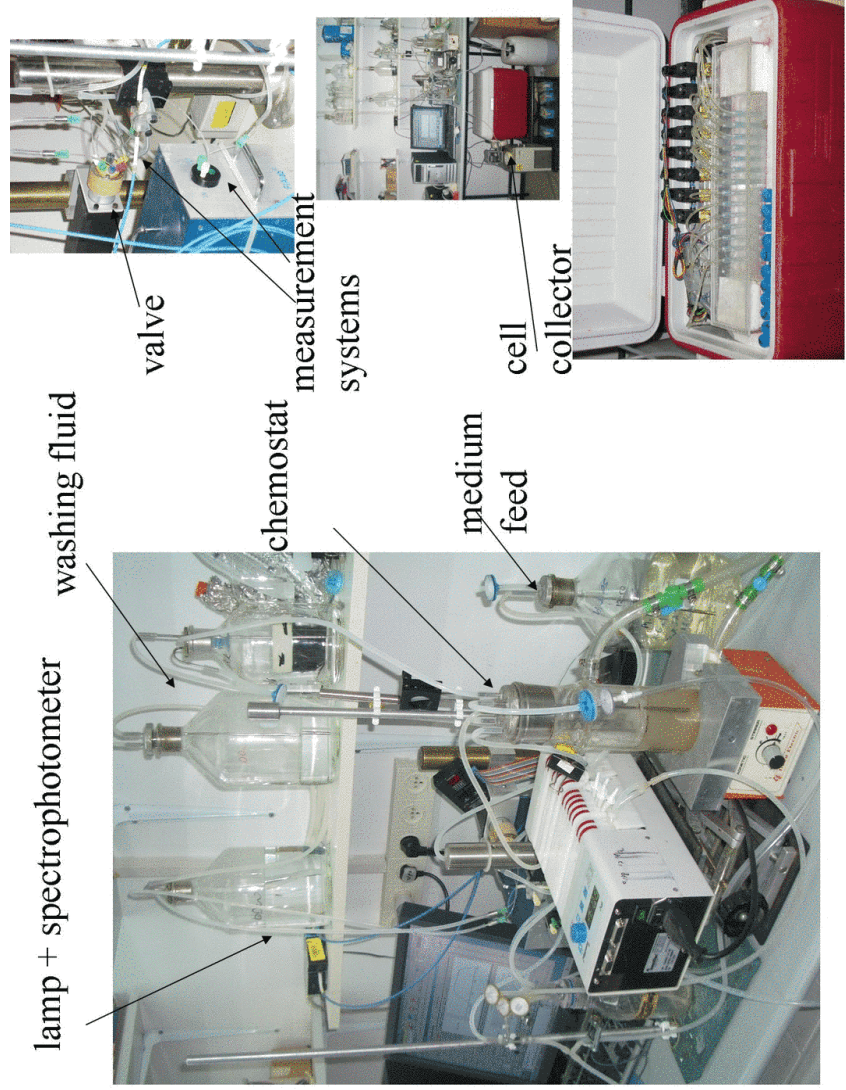
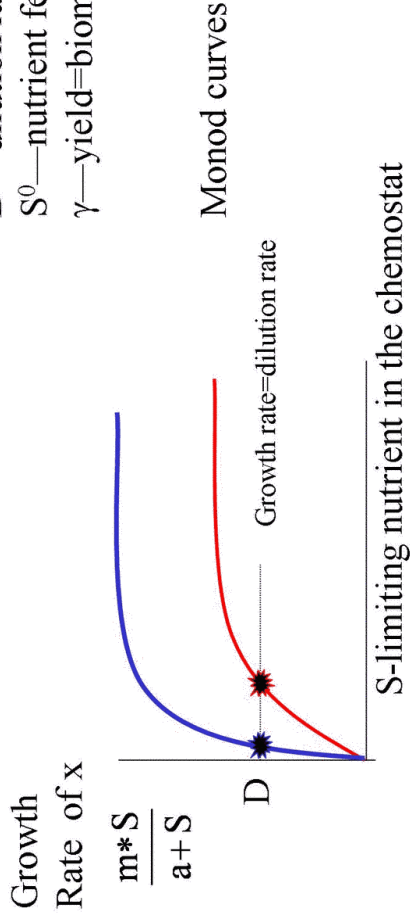


chemostat dynamics

$$\frac{dx}{dt} = x \left[ \frac{m \cdot S}{a + S} - D \right]$$

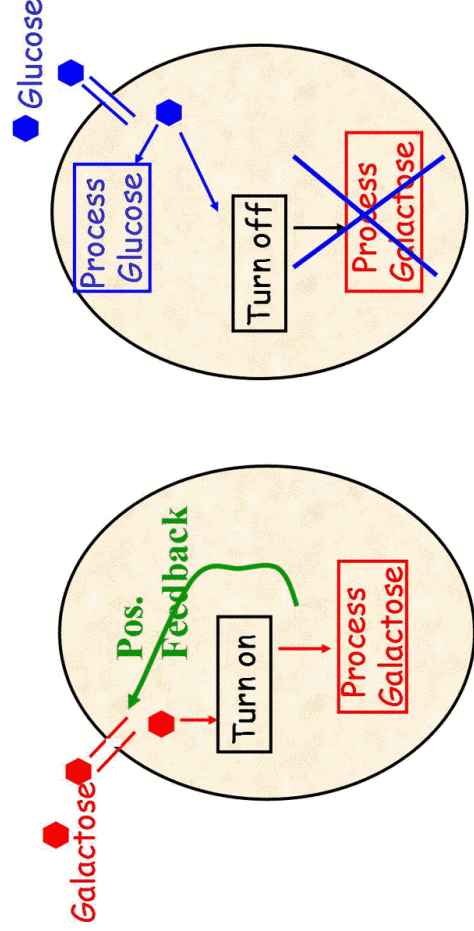
$$\frac{dS}{dt} = (S^0 - S) \cdot D - \frac{x}{\gamma} \cdot \left( \frac{m \cdot S}{a + S} \right)$$

D—dilution rate  
 S<sup>0</sup>—nutrient feed  
 γ—yield=biomass/nutrient





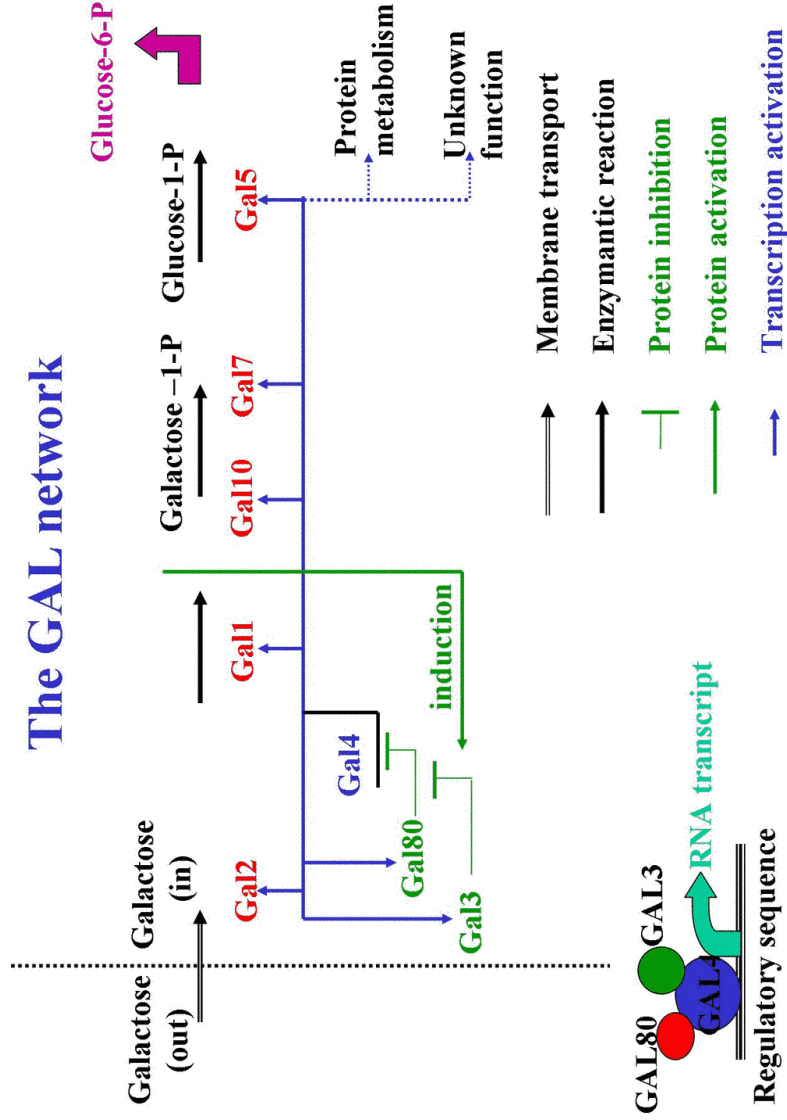
The GAL system:  
Regulation of Galactose utilization



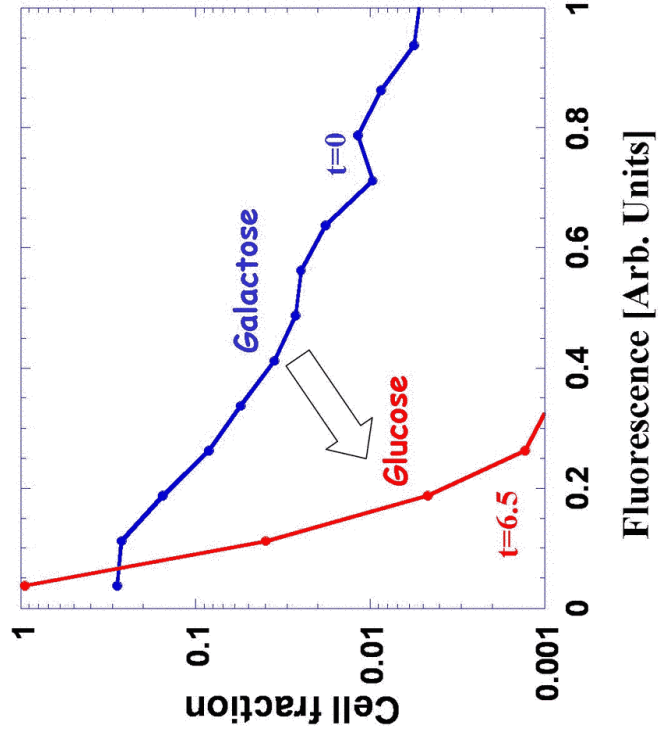
PURE GALACTOSE: ON

PURE GLUCOSE: OFF

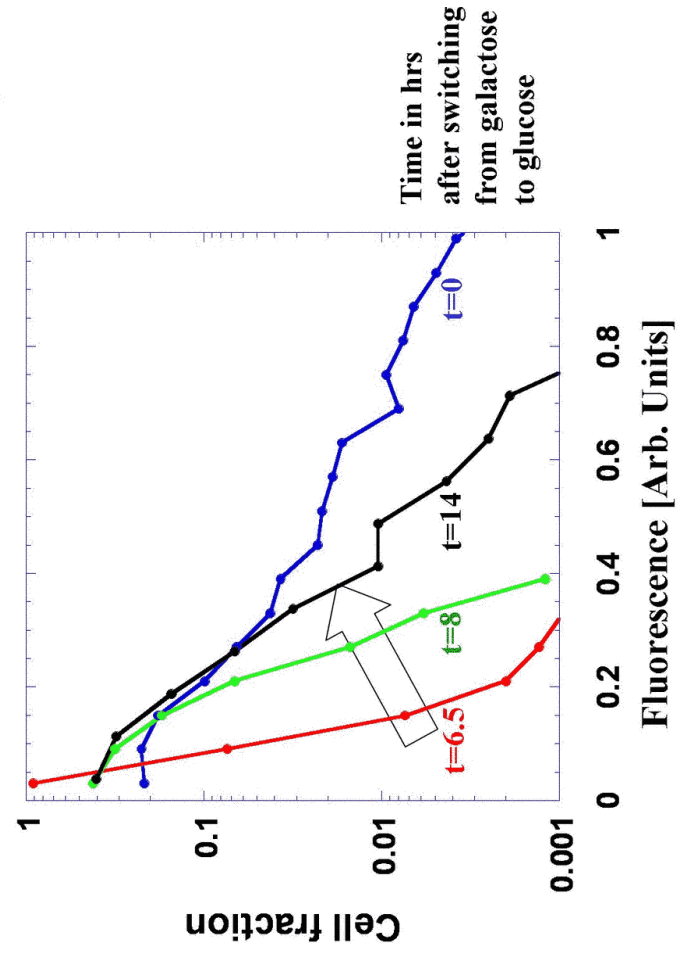
Glucose represses the network by several mechanisms.



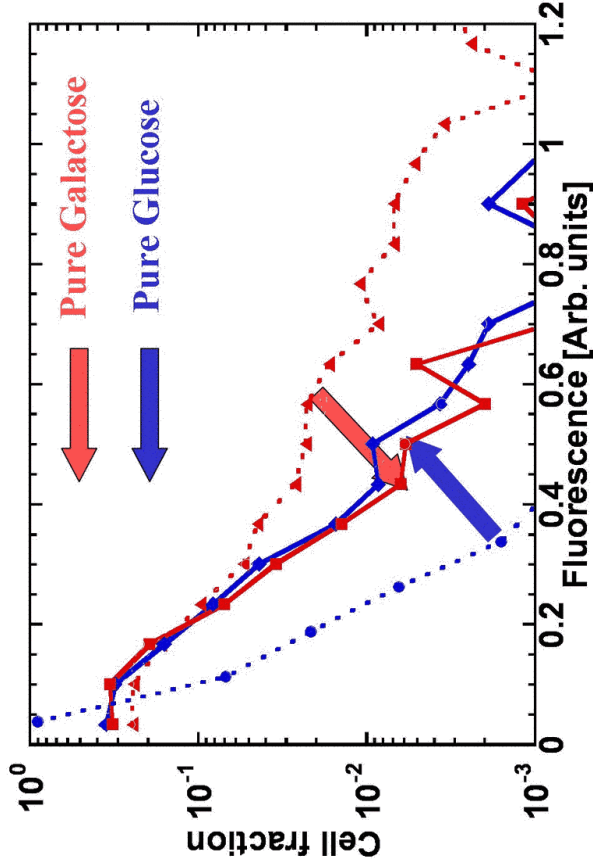
## Glucose repression of the GAL network



## But.... after a few generations the GAL system adapts!



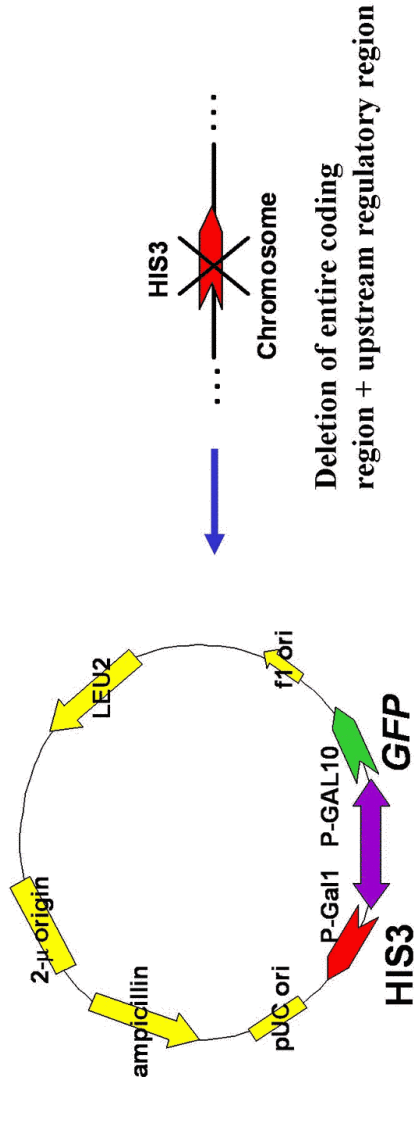
**Invariant Steady-State  
(galactose and glucose in excess)**



Braun & Brenner Physical Biology 2004.

**Gene recruitment experiment:**

**What would happen if we recruit an arbitrary essential gene to the GAL system?**

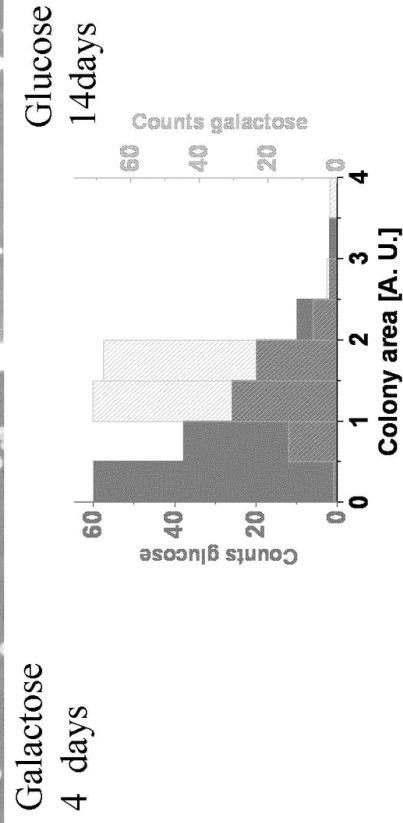
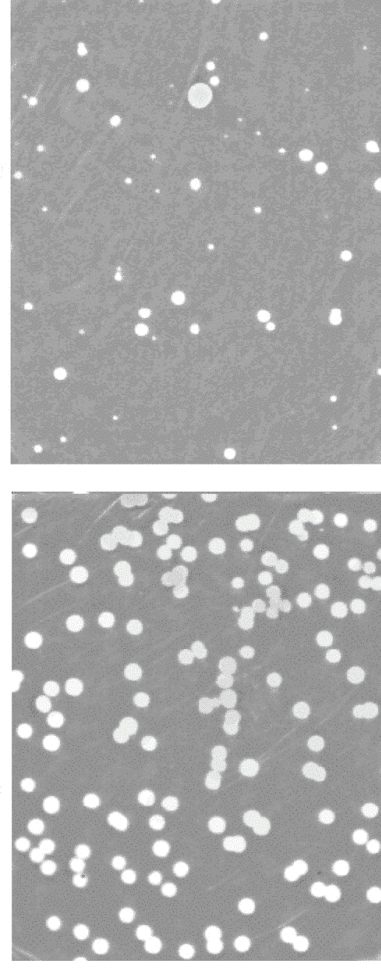


*HIS3*—essential enzyme in the *histidine* pathway

## Challenges of gene recruitment in media lacking *histidine* where *HIS3* is essential

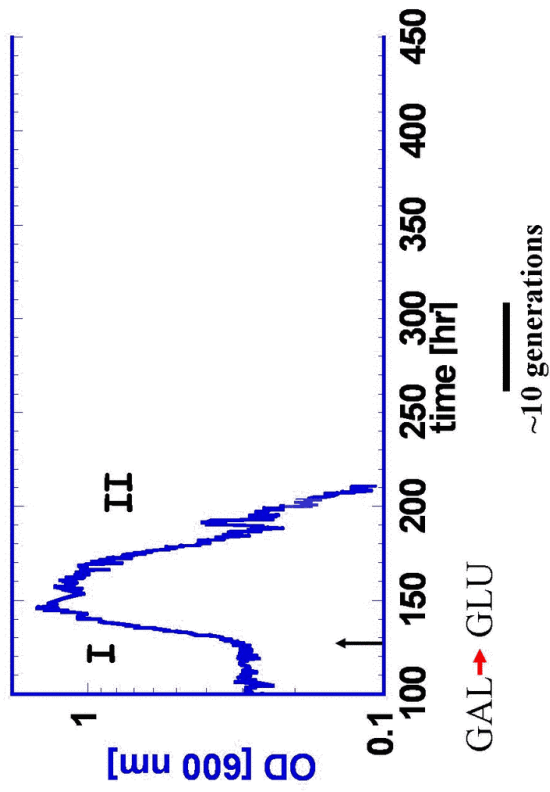
- In pure galactose: the GAL system must simultaneously support regulation of two metabolic tasks, *galactose* utilization and *histidine* biosynthesis (**multitasking**).
- In pure glucose: *HIS3* is **repressed**.
- The dynamic range of the GAL system (1000 fold) is much larger than the amino-acid regulation (a few fold), so *HIS3* can be **over- or under-expressed** beyond its normal levels.
- The recruited *HIS3* is detached from its **natural feedbacks**, limiting its response to relevant inputs.

## Sensitivity to carbon source (medium lacking histidine)



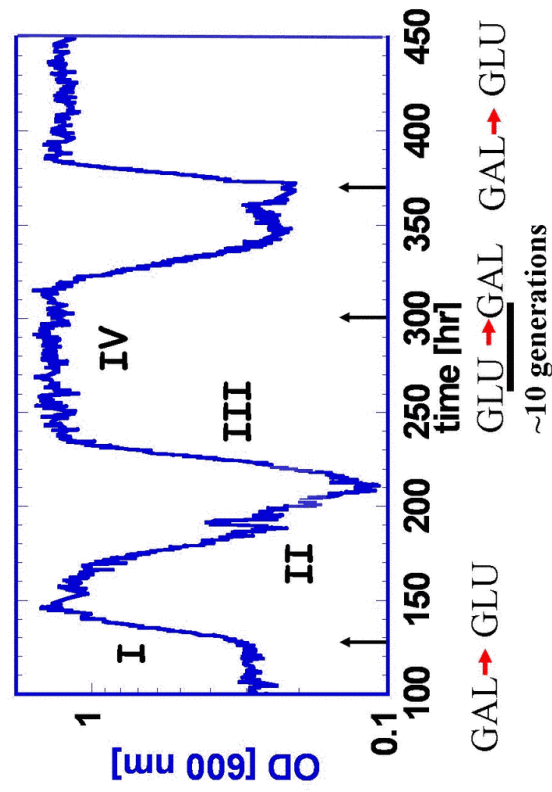
### Galactose-Glucose transition in the chemostat

Online measurement system

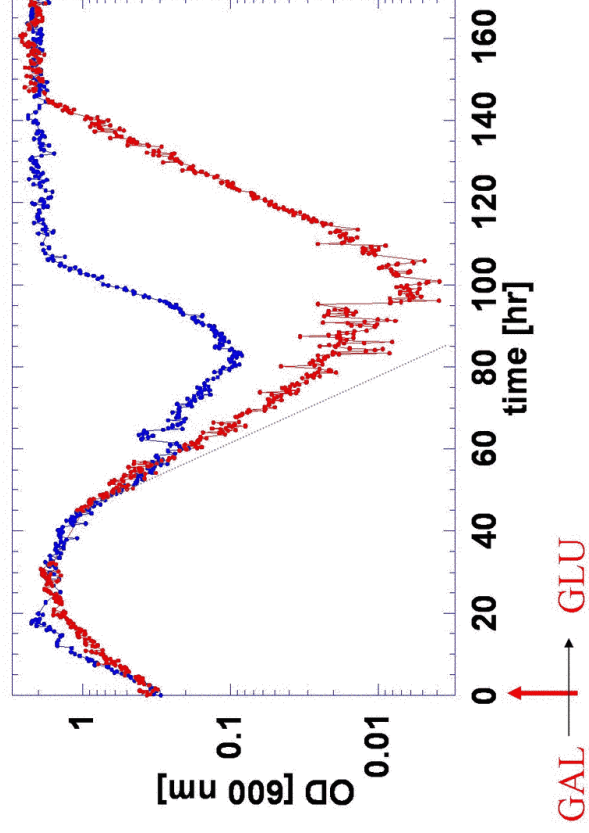


### Galactose-Glucose transition in the chemostat

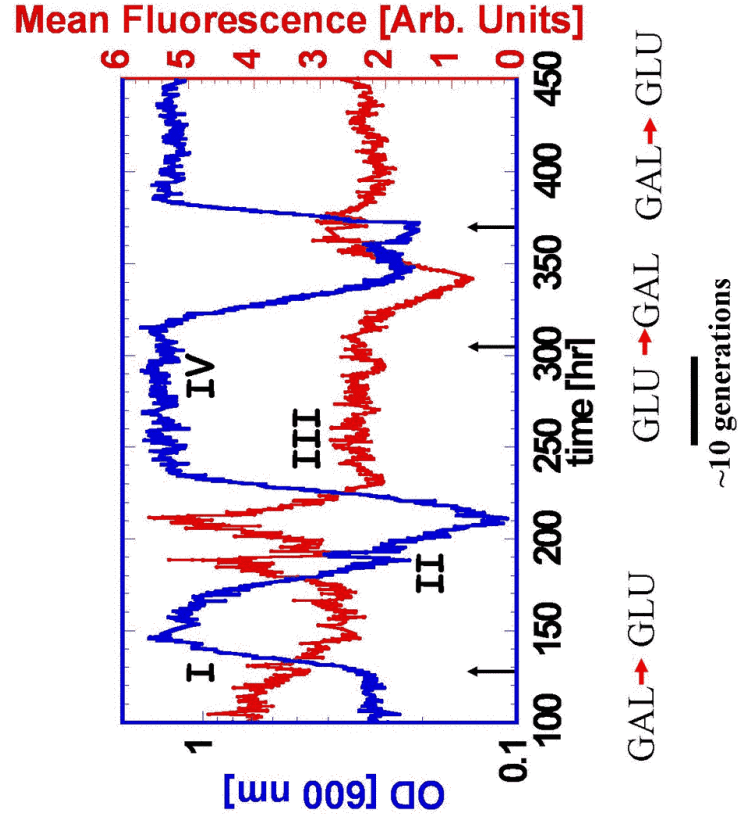
Online measurement system

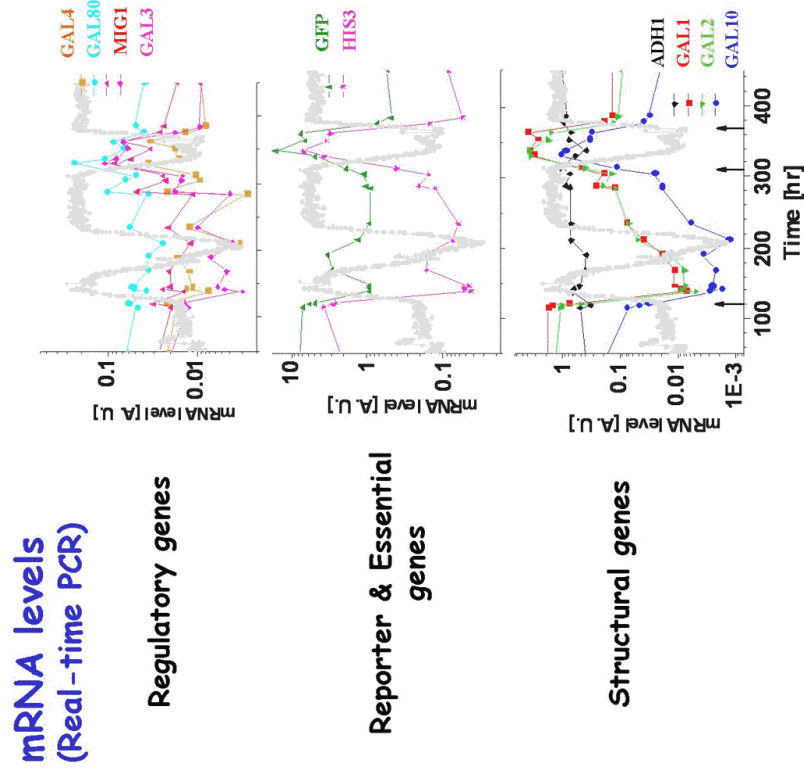


Repetitions—comparing transition to glucose at  $t=0$   
of two separate experiments



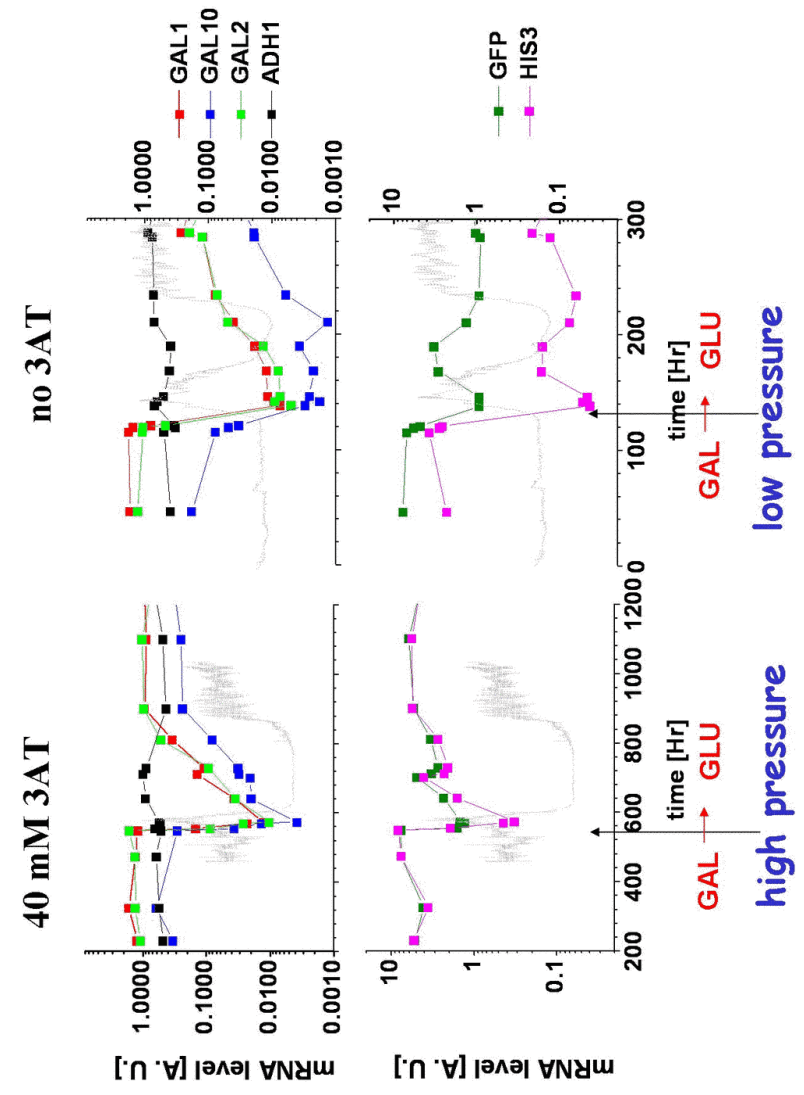
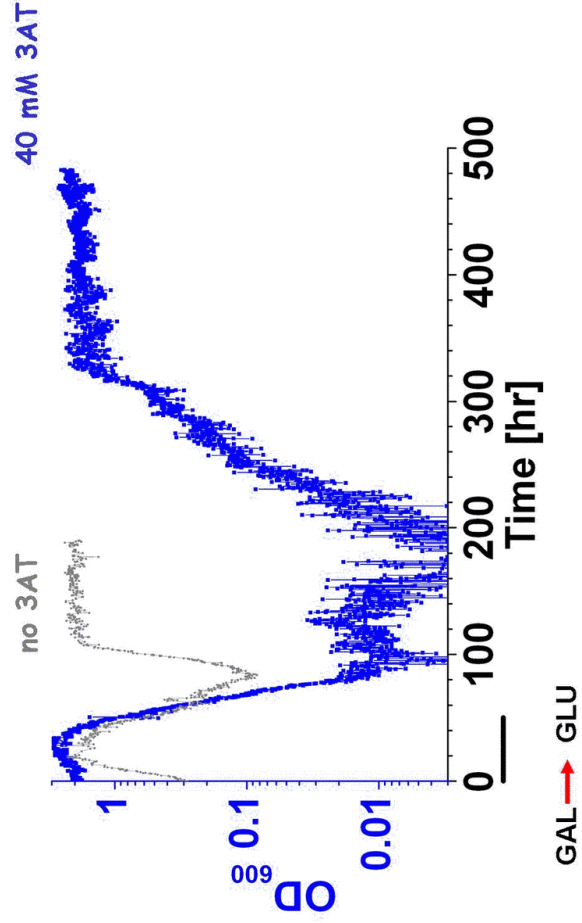
Adaptation is essentially regulatory





**Increasing selection pressure:**

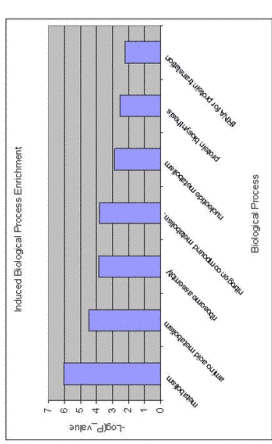
**Introducing 3AT :  
a competitive inhibitor of  
*His3***



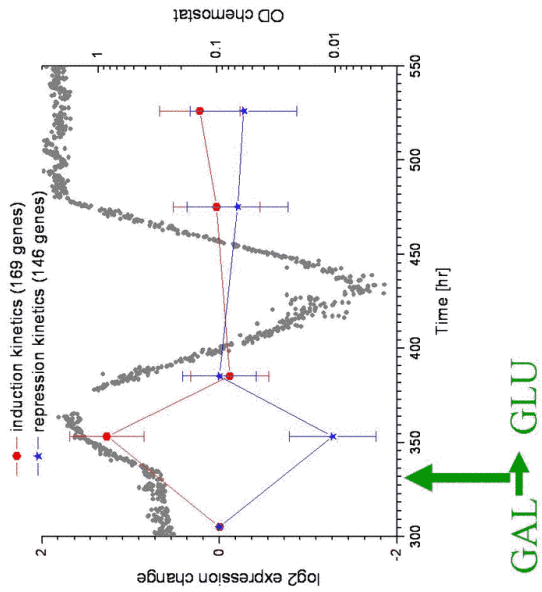
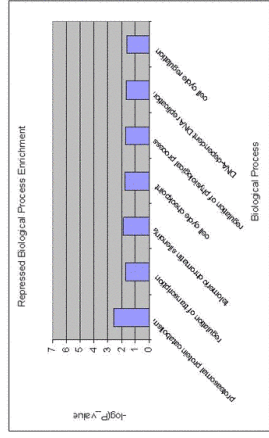


## Array experiments: adaptation is a global phenomenon

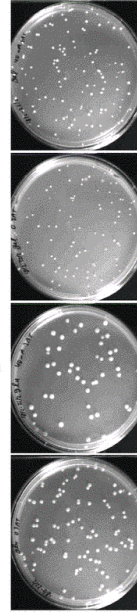
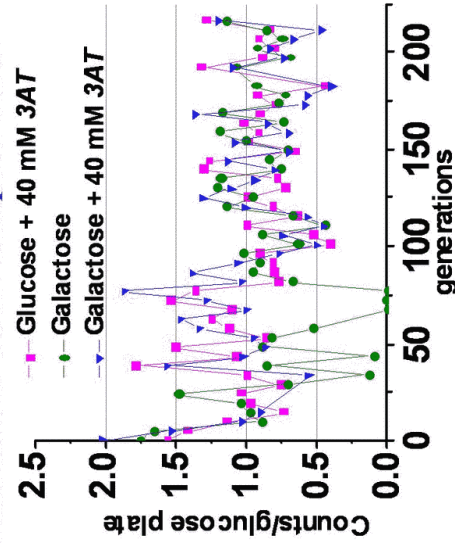
Metabolism;  
amino-acid and nucleic-acid biosynthesis



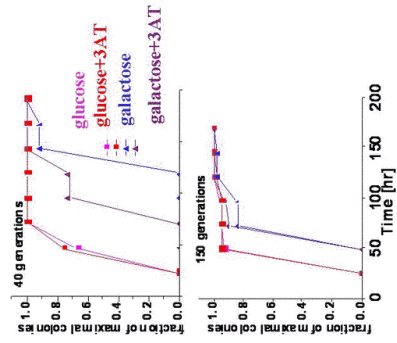
Regulatory, unknowns



## Maintenance of the adapted state



Glucose 0 3AT    Glucose 40 mM 3AT    Galactose 0 3AT    Galactose 40 mM 3AT



Chemostat switched from glucose (after s.s was established) to galactose at  $t=0$ .  
Plates are plated each day with the same cell sample.

# Mating

diploid  
*a/α*

=

haploid  
*α*-type

+

haploid  
*a*-type

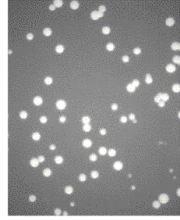
adapted to  
glucose



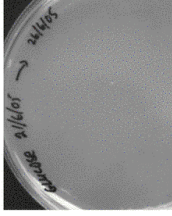
naive



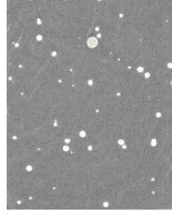
?



narrow size-distribution  
within 2-days

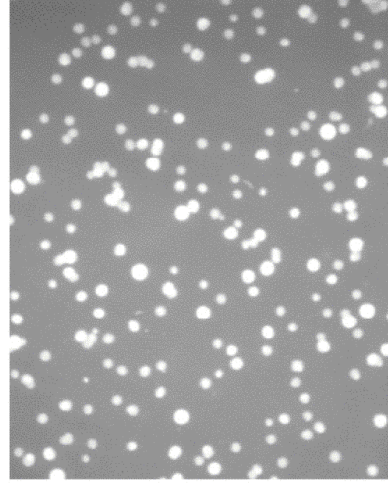


no colonies  
after 5 days

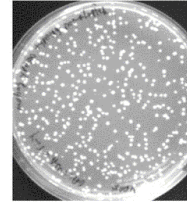


exponential  
size-distribution  
after 14 days

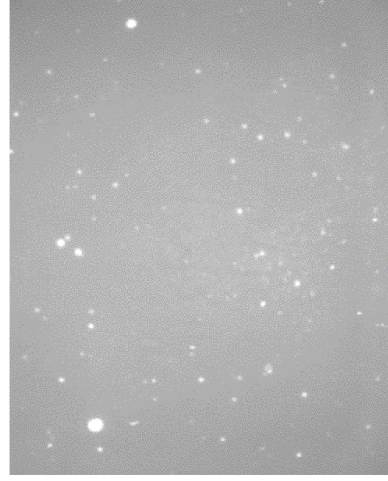
## Diploids: mating of adapted cells, after 5 days



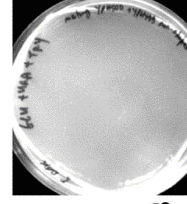
galactose



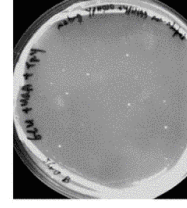
control:  
mating of non-adapted cells  
The same as naïve haploids



glucose



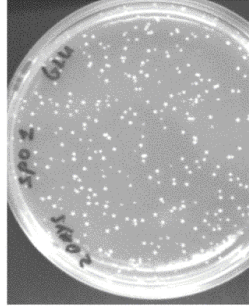
5 days



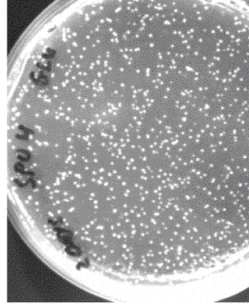
9-10 days

## Sporulation and meiosis

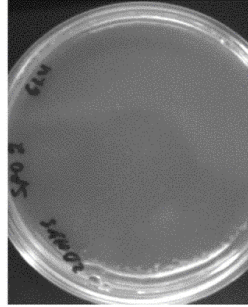
preliminary results of tetrad analysis



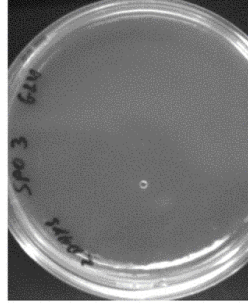
spore 1



spore 4



spore 2

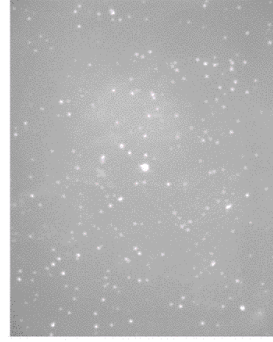


spore 3

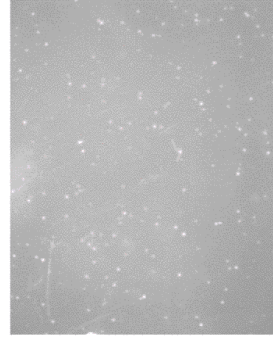
glucose plates after 2 days

**but.....wait!**

after 5 days...well established colonies in the two other cells  
...the same growth characteristics as the diploids

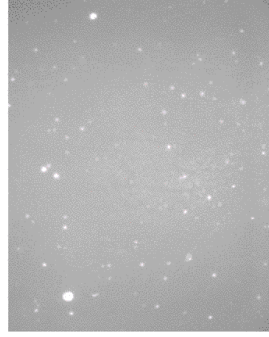


spore 2



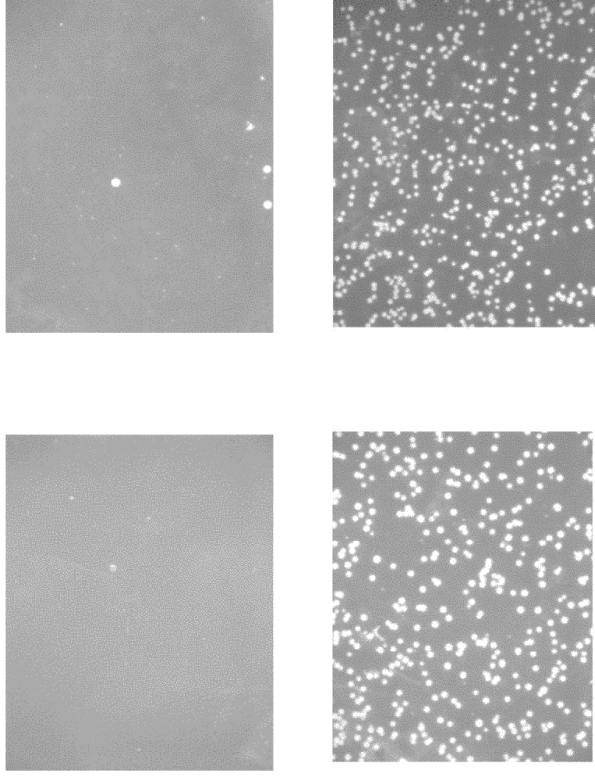
Spore 3

diploids-after 5 days



Adaptation to glucose is a **quantitative** phenomenon in nature  
rather than **“all or none”**.

Another tetrad, after 2.5 days of growth on glucose plates



## Summary

**Genetic regulatory networks have the potential to support highly demanding events of gene recruitment:**

1. Long-term adaptation of gene regulation, induced by an environmental switch, sets a new cell state which is fixated in the population by, yet unknown, mechanism.
2. Gene recruitment exposes the great plasticity of genetic regulatory systems and their potential to deal with unforeseen situations (evolvability potential); the regulatory system reprogrammed to precisely tune the expression of a foreign gene.
3. The approach presented provides a step in making gene recruitment the subject of experimental investigation, promising to extend our understanding of genetic regulatory systems and their evolution.

## Adaptation mechanism: genetic or non-genetic?

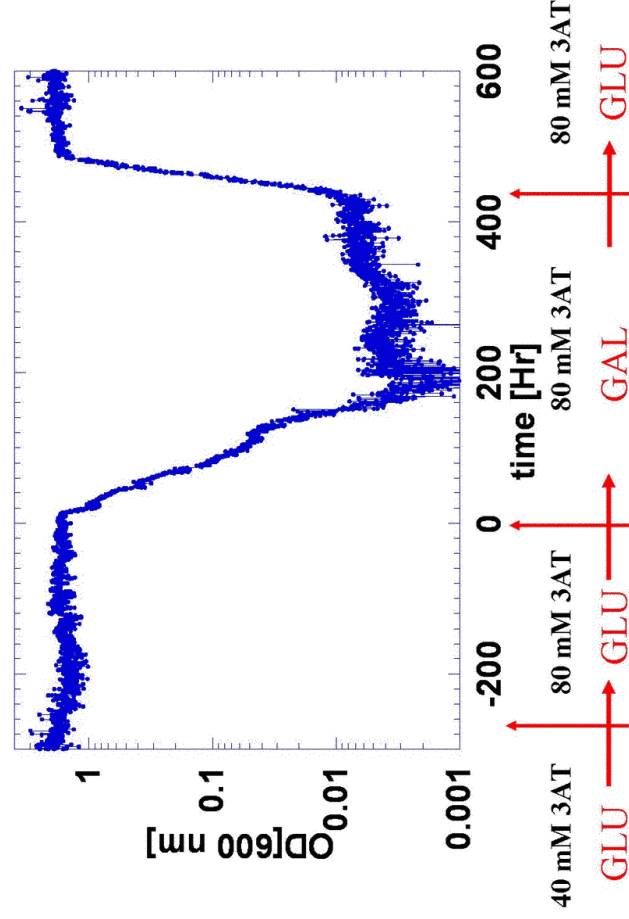
1. Genetic mutation/s followed by selection.
2. Epigenetic inheritance; chromatin modifications, factors...
3. A mixture of genetic plus epigenetic mechanisms.
4. Lamarckian process; inherited useful modifications induced by the environment.

Recall some facts:

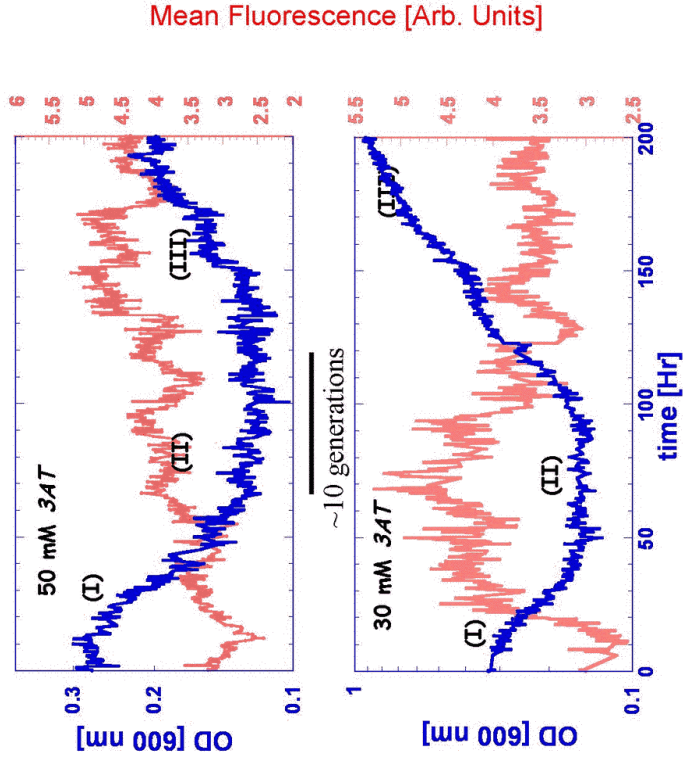
- On plates, all colonies could grow—no selection of a rare subpopulation.
- In chemostat, adaptation is fast ( $\sim 10$  generations) and occurs only after a medium switch. The dynamic trajectory is predictable.
- Mating shows that adaptation is a quantitative phenomenon in nature.
- Following meiosis, two cells exhibit strong adaptation, two intermediate levels of adaptation (similar to diploid) and high variability.

### Multitasking due to gene recruitment:

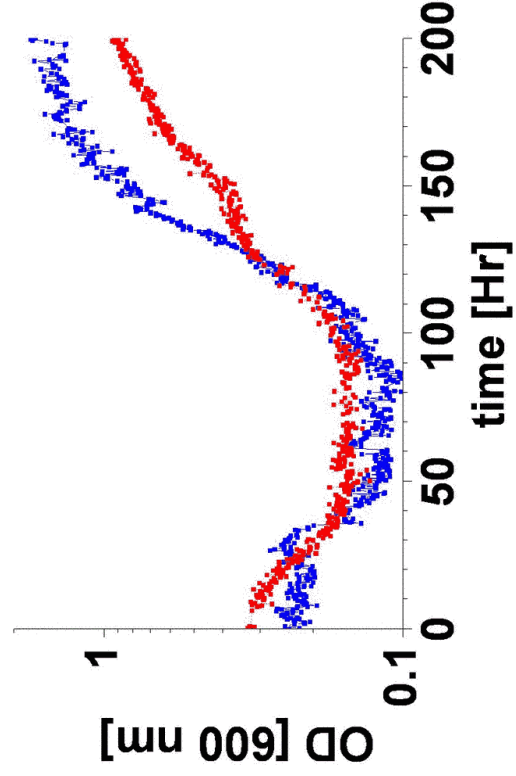
In galactose: the GAL system needs to support both *galactose* utilization and *histidine* biosynthesis.



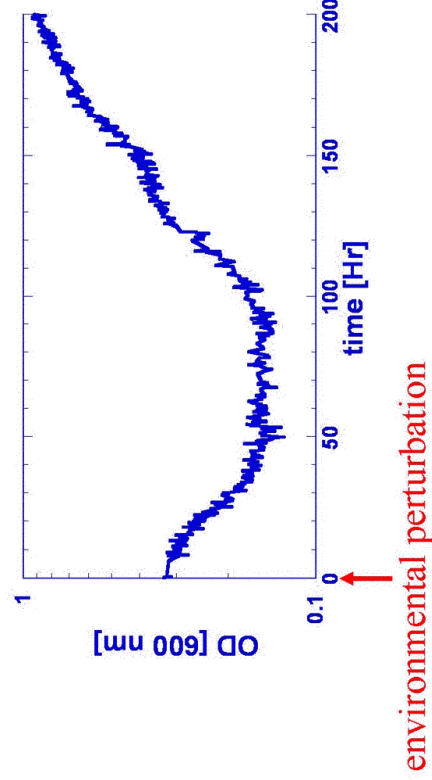
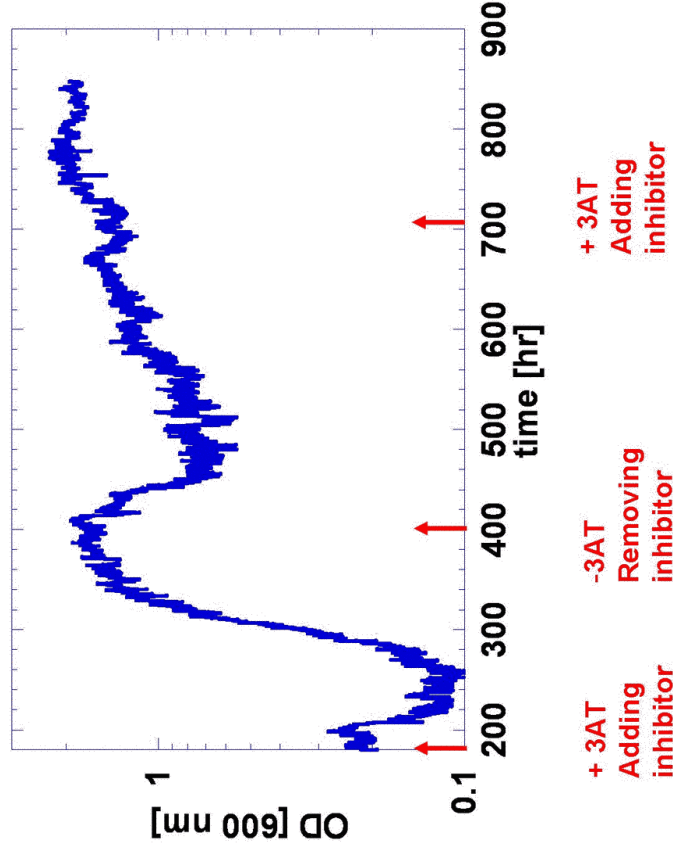
**Adaptation in pure galactose medium**



**Repetitions—introducing 30 mM 3AT at t=0  
in two separate experiments**



**Adapted state is attractive (homeostasis)**



Two possible interpretations:

- Genetically heterogeneous population + selection: simple calculation shows that “rare cells” cannot lead to a better fitted population in the short time scales involved.
- Fixation of a new phenotype (cell state) in the population—**phenotypic inheritance.**

## A model of two subpopulations

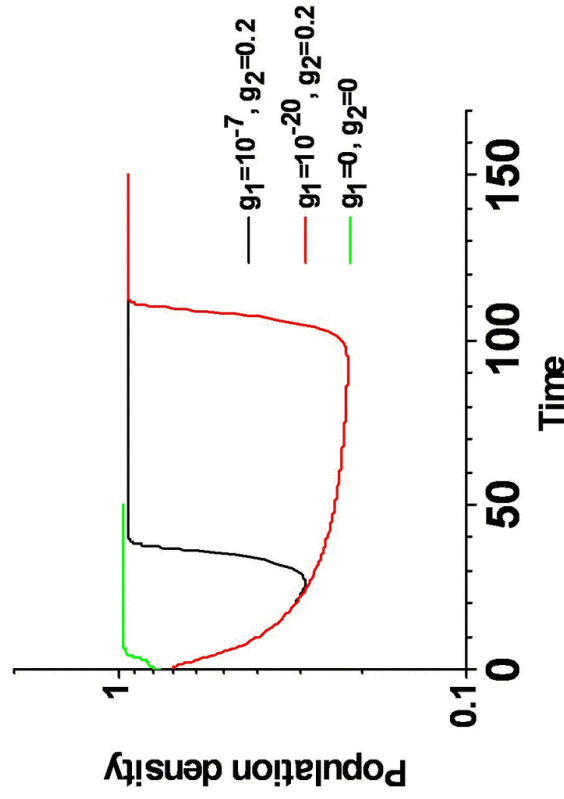
- An environmental perturbation causes cells to explore their regulatory/metabolic phase-space.
- A new metabolic (phenotypic) fixed point is reached—cell individuality causes each cell to get to that new point at its own rate.
- The new phenotype is inherited and better fitted to the environment and thus fixated in the population.  
**A different cell population is established in the chemostat.**

$$\frac{dx_1}{dt} = x_1 \left[ \left( \frac{m_1 S}{a_1 + S} \right) - 1 \right] - g_1 x_1 + g_2 x_2$$

$$\frac{dx_2}{dt} = x_2 \left[ \left( \frac{m_2 S}{a_2 + S} \right) - 1 \right] + g_1 x_1 - g_2 x_2$$

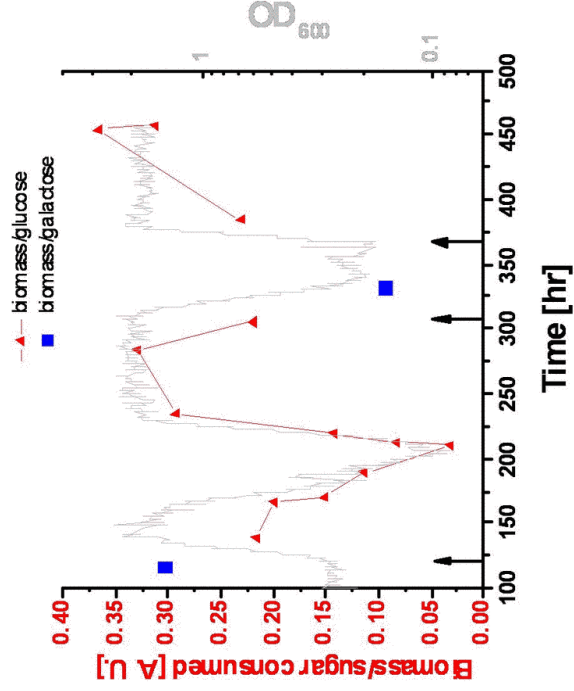
$$\frac{dS}{dt} = 1 - S - x_1 \left( \frac{m_1 S}{a_1 + S} \right) - x_2 \left( \frac{m_2 S}{a_2 + S} \right)$$

State 2 is better fitted to the new environment.

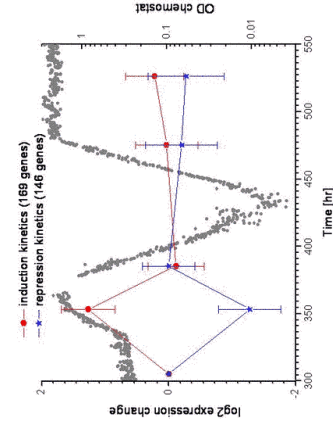




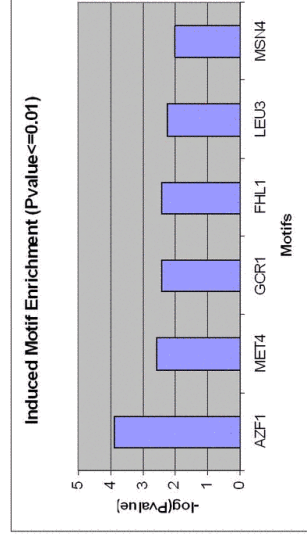
# Metabolic yield



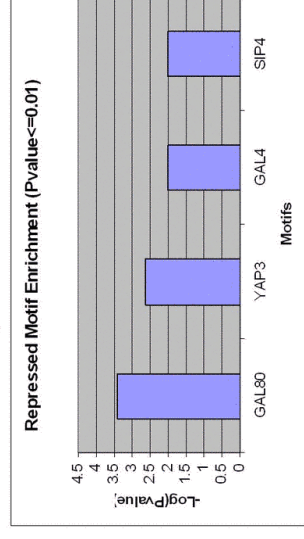
# Array experiments



# Induced motifs

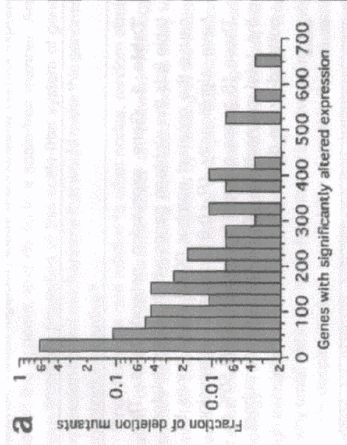


# Repressed motifs



synthetic lethality;  
knockout of pairs of genes

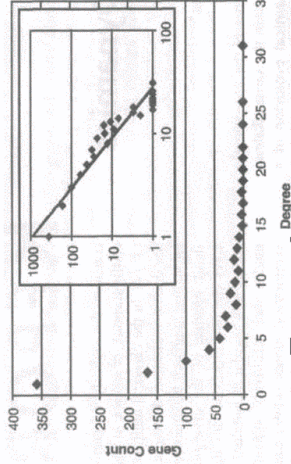
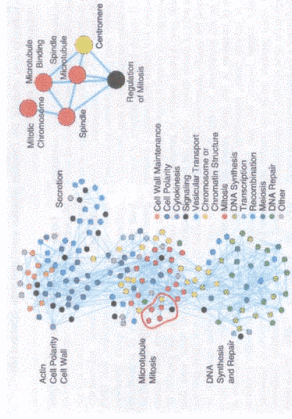
expression profiles;  
knockout of a gene



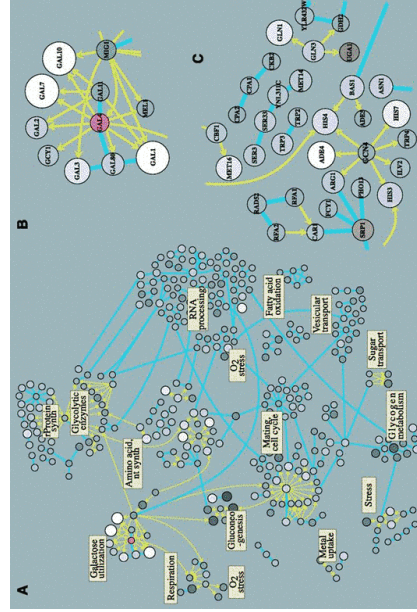
**Featherstone and Broadie**  
Based on Hughes et al  
*Cell* 2000.

**In Yeast:**

- **Gene dispensability: ~73% of the genes are nonessential.**
- **Gene deletion causes a major rearrangement of expression patterns.**
- **Synthetic lethality reveals massive cross-talk between functional modules.**

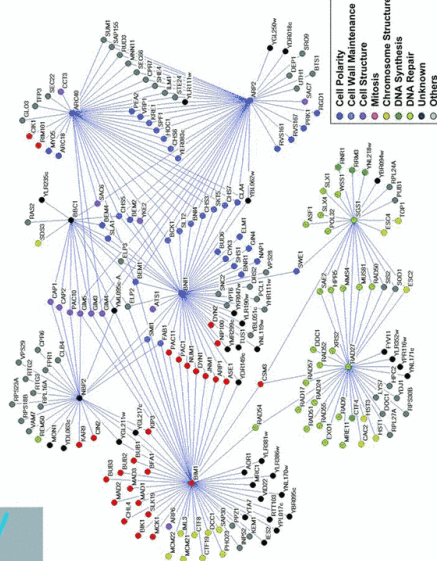


Tong et al,  
*Science* 303, 808 2004



*Science* 294, 2364, 2001

Active “reprogramming” of transcription  
ref: Kafri, Bar-Even & Pilpel  
*Nature Genetics* 37, 295, 2005.



*science* 292, 929 2001.



## The need for a population view of gene regulation

- non-genetic individuality.
- Intracellular dynamics longer than cell-cycle; protein inheritance along generations.
- Long-term population adaptations.

Population history and epigenetic inheritance play important role in determining expression patterns.