Protein Distributions
in
Dividing Cell Populations

(work in progress)

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Outline

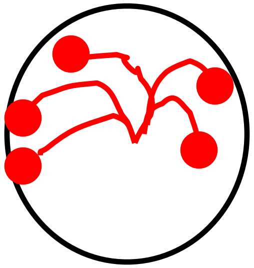
* Background & problem definition
* Experiment design: the search for universality
* Experimental results and analysis
* Scaling of the time-dependent distributions – an analogy with fragmentation theory
* Conclusions for dividing cell populations

Phenotypic variation

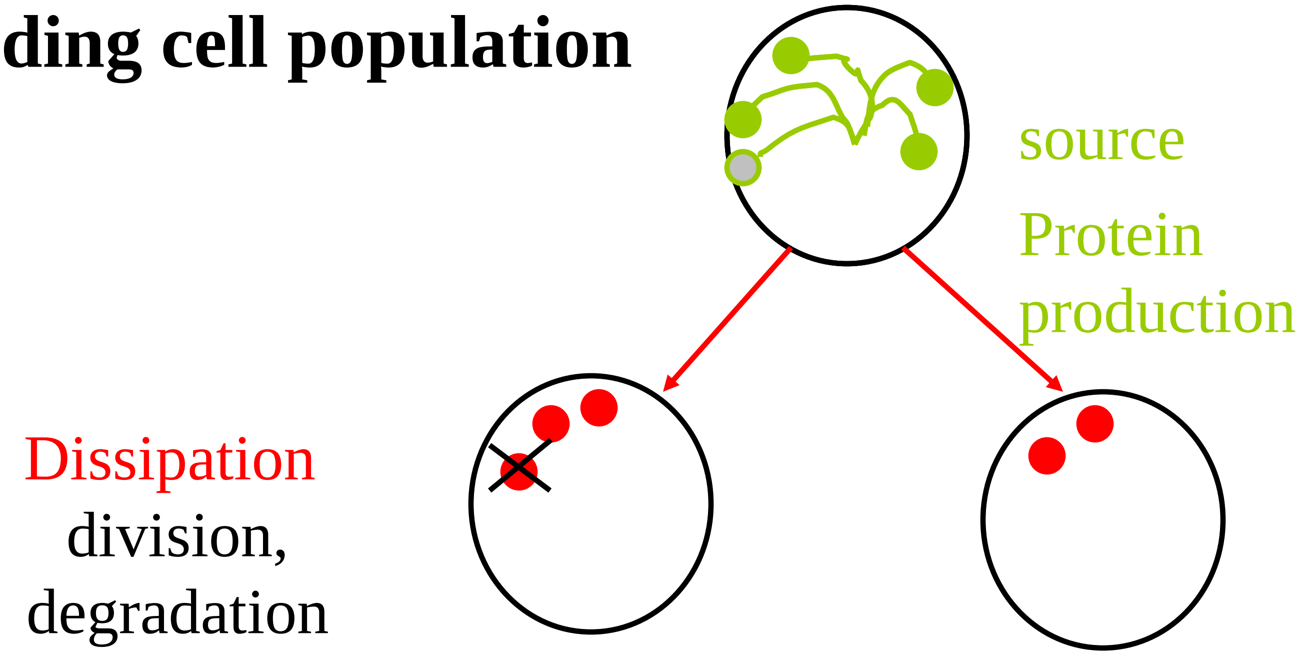
* In a population, each individual is unique, genetically as well as phenotypically

* Clonal microorganism populations provide a model system to isolate the phenotypic component of variation. In particular, protein content is of interest.

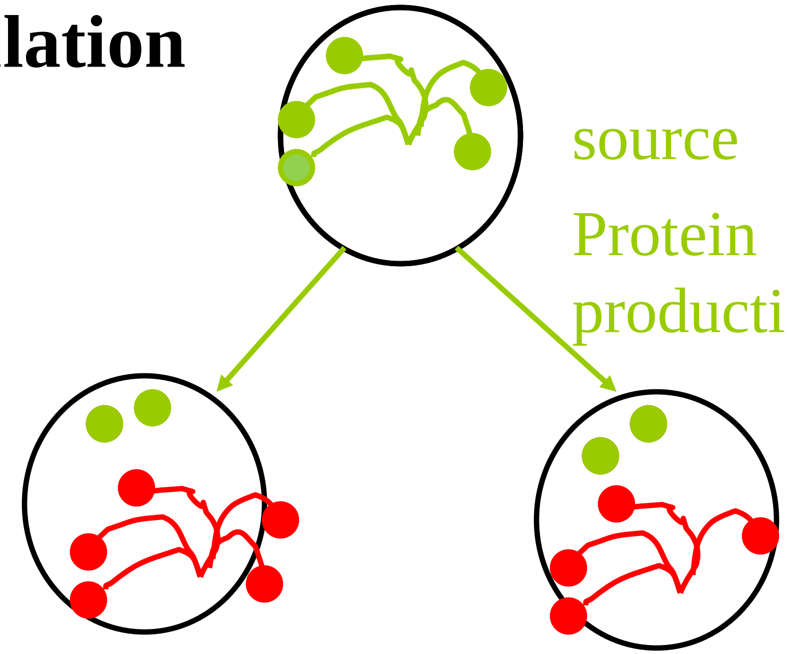
Protein distribution in a dividing cell population



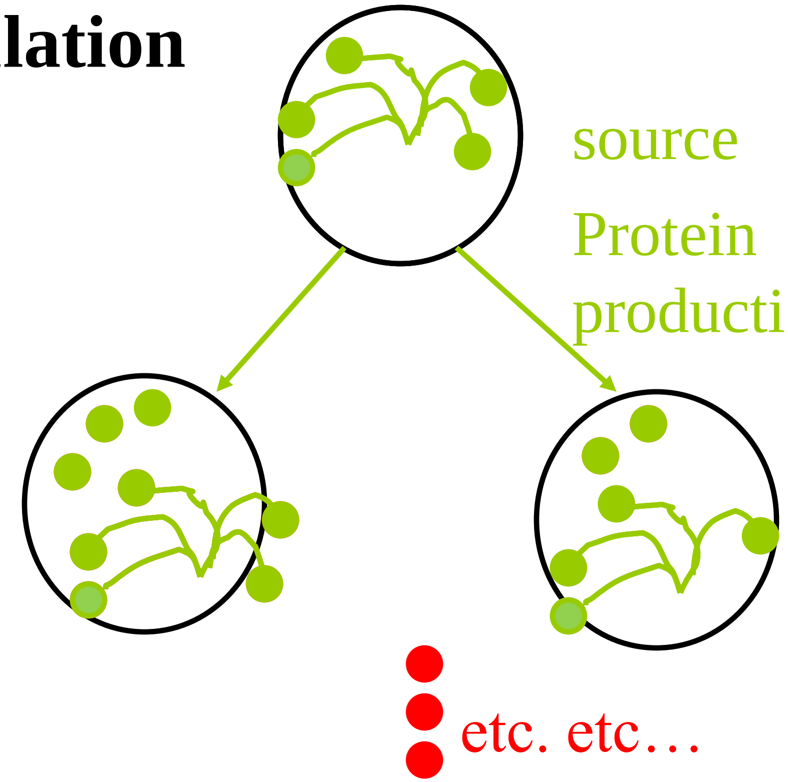
Protein distribution in a dividing cell population



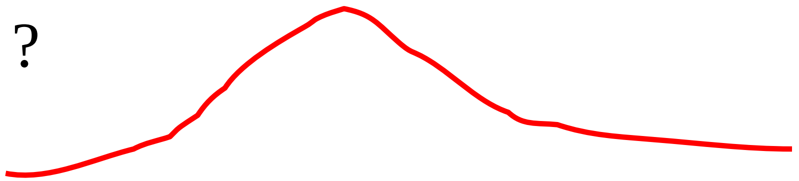
Protein distribution in a dividing cell population



Protein distribution in a dividing cell population



Distribution in population ?



Processes in the population:

* Protein production: regulated – but stochastic
temporally discrete bursts

* Protein degradation
* Cell division: Also temporally discrete
occurs at random times
variation in division proportions

Characterizing the processes

* Experiment: Steady states are universally characterized by broad distributions

often with exponential tails

* Theory: different models can result in the same steady-state distributions, for example:

** Stochastic bursts with continuous dissipation

(Berg, 1979; Paulsson, 2000; Friedman et al 2006)

** Deterministic production with random division times (Friedlander & Brenner 2008)

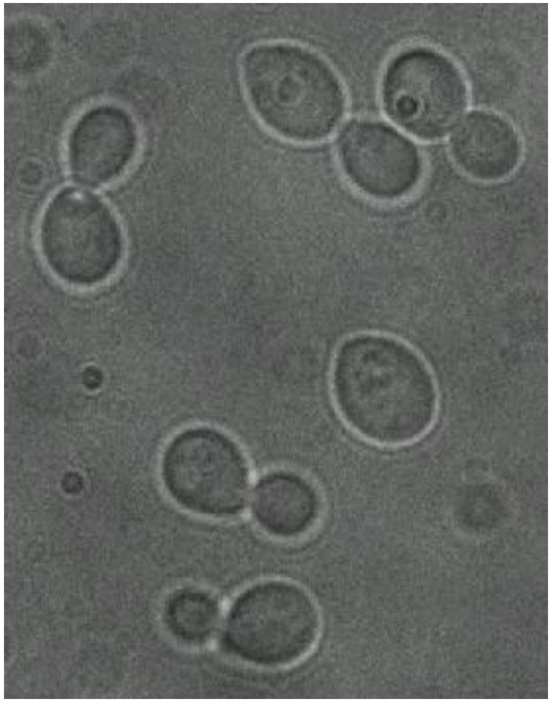
Characterizing the processes

To uncover the processes underlying variation, perturbations need to be applied to the population and the distribution dynamics measured quantitatively.

Additionally, the degree of universality is of interest: are the processes and distributions specific to genes, organisms, experimental conditions?

In search of Universality: bacteria and yeast – A comparative study

S. cerevisea



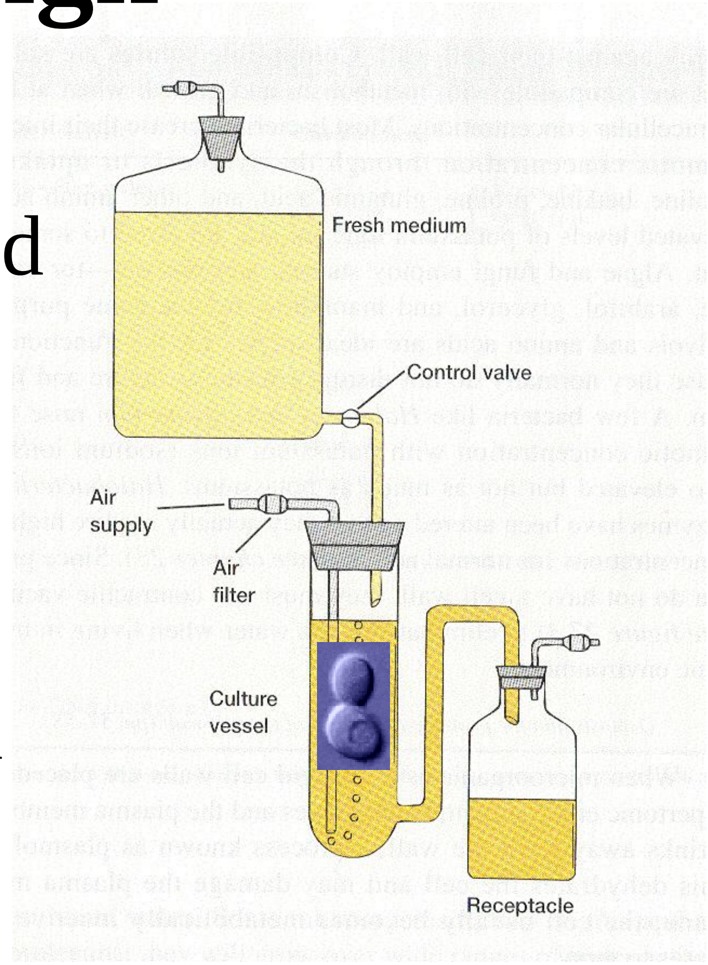
Eukaryotic organism
Typical size ~5 μm
Division by budding

E. coli

Prokaryotic microorganism
Typical linear size ~1 μm
Division by fission

Experiment design

* Grow microorganisms in continuous culture – controlled conditions for long times
* Measure distributions of fluorescent protein (gfp) expressed from a metabolical inducible/repressible gene



Collaboration: Erez Braun, Technion;
Hanna Salman, Pittsburgh
Albert Libchaber, Rockefeller

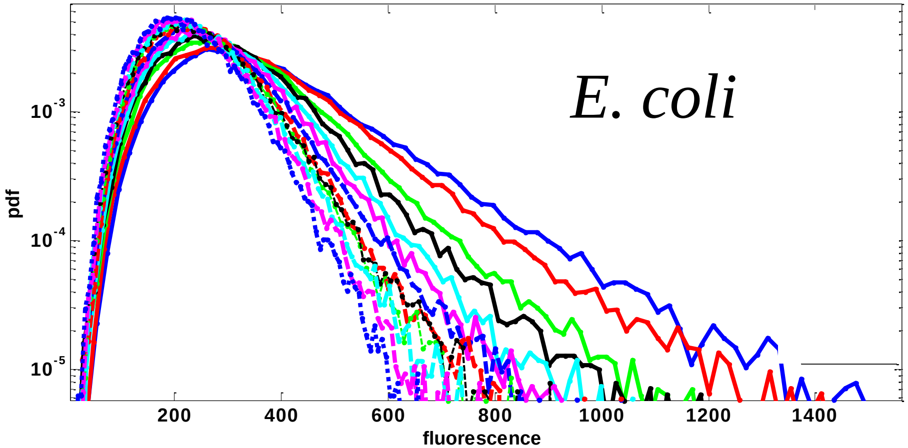
First results – dilution operator

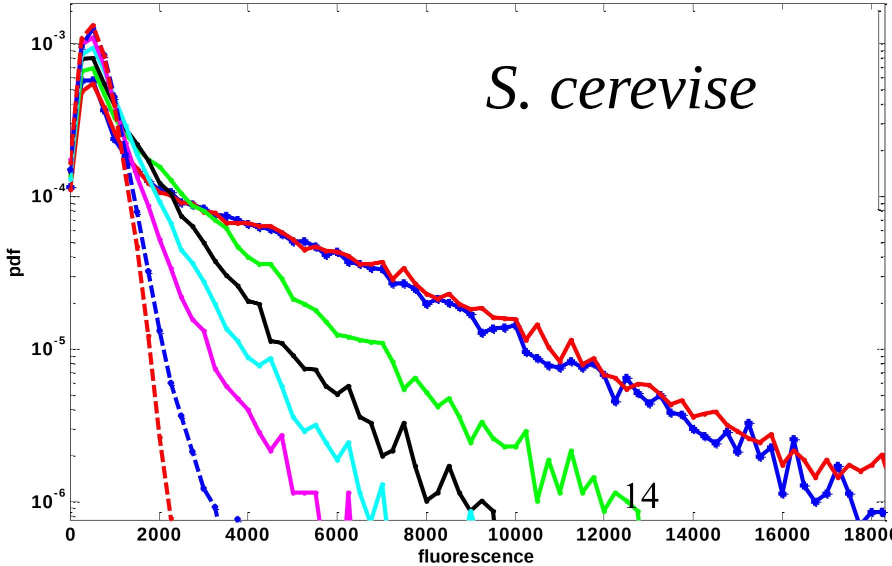
To separate out the action of the dilution/dissipation processes, grow culture to steady-state expression and switch to repressing conditions. To a good approximation the gene expression (the production process) turns off immediately. Then we can follow the other processes.

GFP reporters measured from the GAL system (yeast) and the LAC system (bacteria), analogous – but different – metabolic expression systems, under metabolically functional conditions.

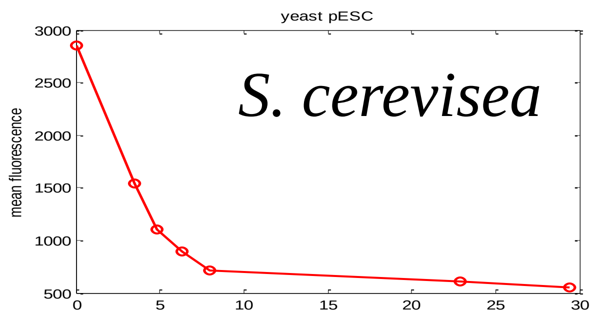
Results of comparative experiments: Distributions

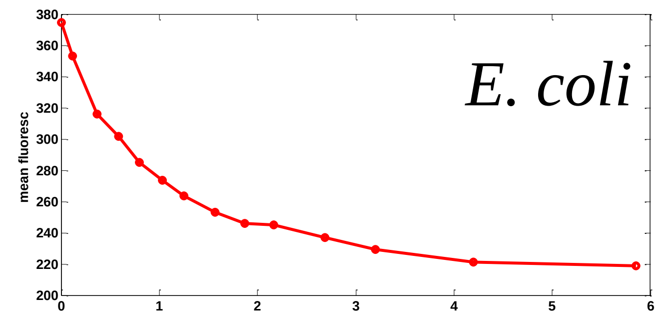
Distribution shapes along time:
* Exponential tail is retained through dynamics
* Yeast have higher copy number - different head shape

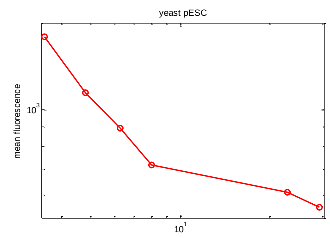


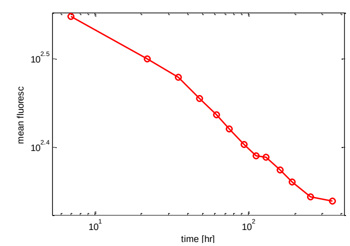


Results of comparative experiments: Moments vs. time









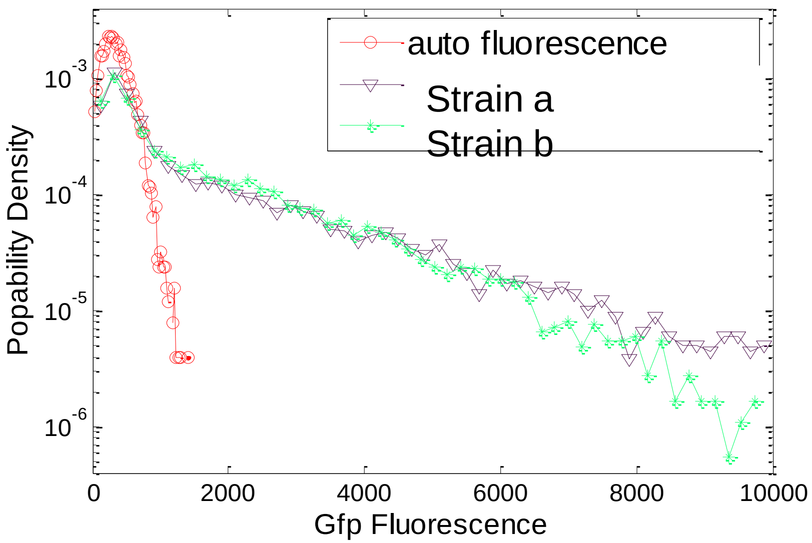
Mean and variance along time decrease slower than exp.

* Protein degradation with a single timescale is not the dominant process

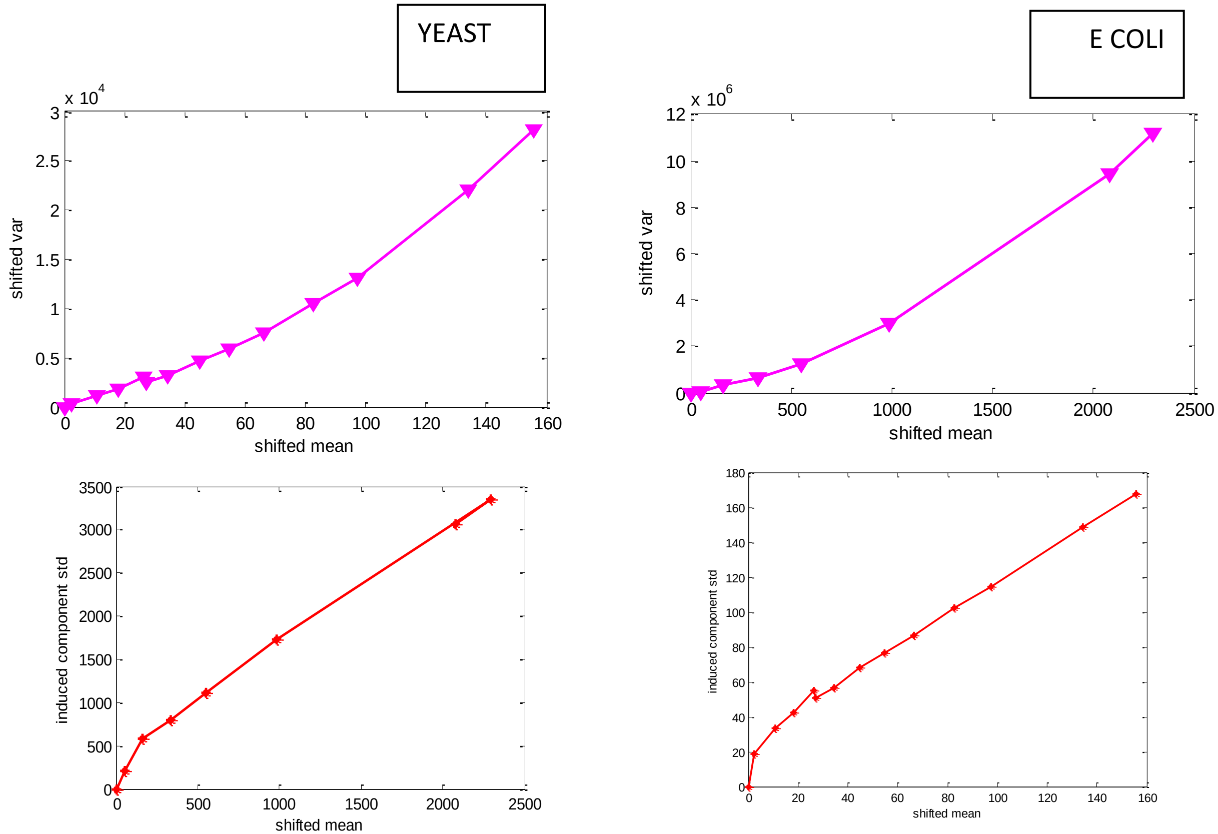
* Possibly dominated by cell division (consistent with protein lifetime)

Analyzing the moments of the distributions

Total variation = autofluorescence + induced fluorescence
Reasonable assumption: independence of the two RVs



Results of comparative experiments: MomentSs



Conclusion: the induced component in dynamics is dominated by the exponential tails – std linear with mean

Describing cell division by the fragmentation operator

$$\frac{\partial}{\partial t} n(x,t) = -b(x)n(x,t) + 2\int_x^{\infty} b(\xi)n(\xi,t)\frac{d\xi}{\xi} = 0$$

n(x,t) = number density of cells with protein x at time t
mass conservation

b(x) = probability per unit time to divide
Protein content is assumed to divide uniformly between daughter cells.

Fragmentation – a widely-studied problem in physics:
polymer degradation, breakup of liquid droplets…

Technical note: the normalized distribution *f(x,t)* obeys

$$\frac{\partial}{\partial t} f(x,t) = -b(x) f(x,t) + 2\int_x^\infty b(\xi) f(\xi,t) \frac{d\xi}{\xi} - f(x,t)\int_0^\infty b(\xi) f(\xi,t) = 0$$

Generally defining a nonlinear equation, however at balanced growth
The total number of divisions per unit time is constant –

$$\int_0^\infty b(\xi) f(\xi,t) = \mu$$

Leaving the operator still linear.

Scaling theory of fragmentation

(Cheng & Redner 1988)

$$n(x,t) \sim \frac{1}{s(t)^2}\,\phi\left(\frac{x}{s(t)}\right)$$

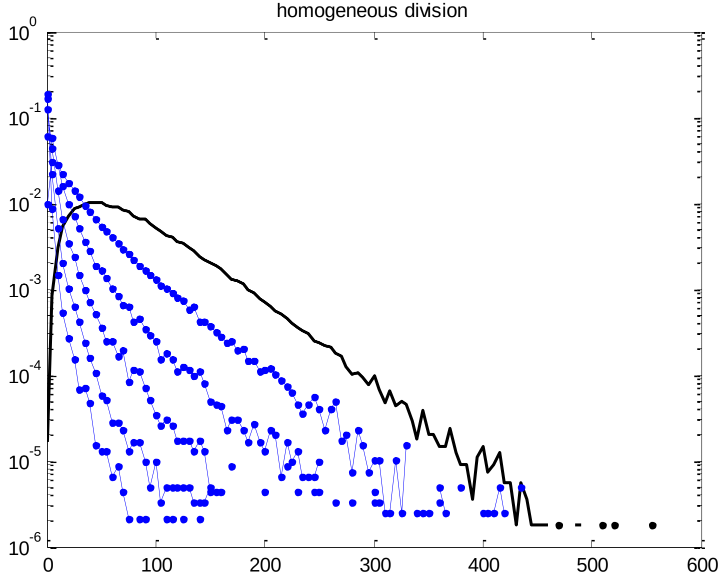
Such a scaling implies, in particular, a trajectory through exponential tails with a time-dependent typical scale.

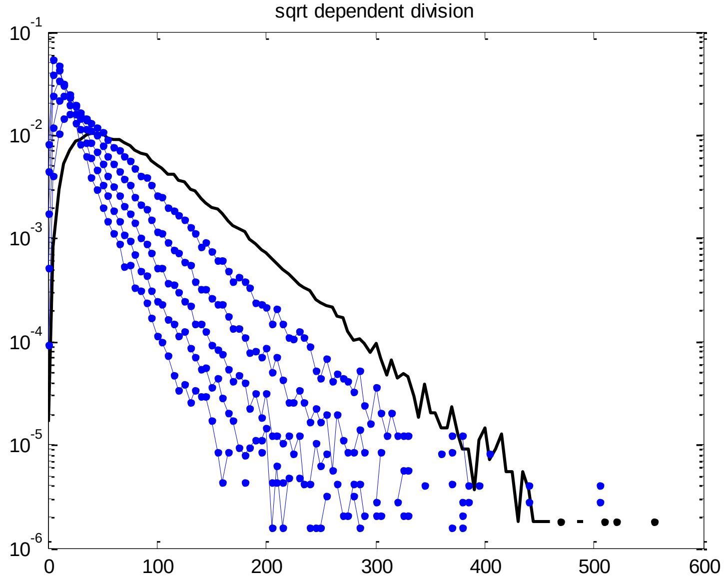
For a class of homogeneous division function, $b(x) \sim x^{\lambda}$
it can be shown that scaling persists only for $\lambda > 0$.

In terms of division in the population this implies a non-uniform (*x*-dependent) probability per unit time to divide.

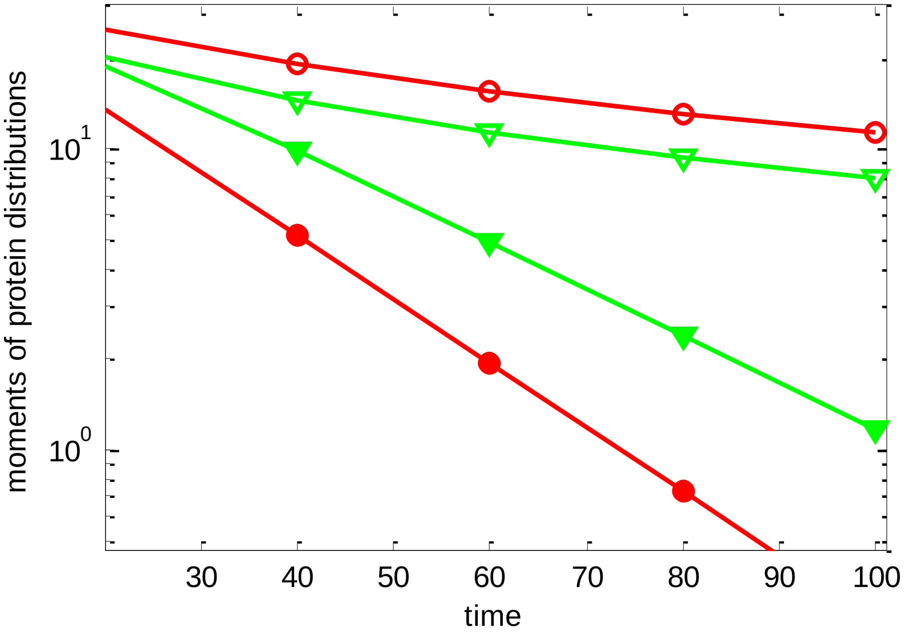
Monte-Carlo simulations confirm this distinction

Scaling is found for non-uniform (x-dependent) division rate.





Non-uniform division is also consistent with non-exponential decay of the moments:



Conclusions

* The comparison between yeast and bacteria reveals population-level universalities although many molecular/cellular details are different.

* This shifts the focus of describing variation from a microscopic-mechanistic point of view to that of the population properties.

* The inducible exponential tail is dominant in determining relations between moments. It exhibits a form of scaling which constrains population-level division processes.

* This scaling implies non-uniform division properties across the population, i.e. variation over several timescales

Further questions

* Transient induction experiments: combine what we have learnt about the dilution operator to constrain properties of the production operator. Preliminary results: data are inconsistent with the popular picture where only stochastic gene expression, and not variation in division, dominate the behavior.
* Preliminary data suggests that a non-uniformity in the population with a relatively long coherence time (>generation) is important for long-term physiological adpatations. What are its inheritance properties?
* Modulating division rates can be an adaptive strategy under challenge (for example growth arrest of subpopulations).

  Measure and decipher distribution dynamics through adaptation to characterize it

Acknowledgement

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Life Science & Engineering

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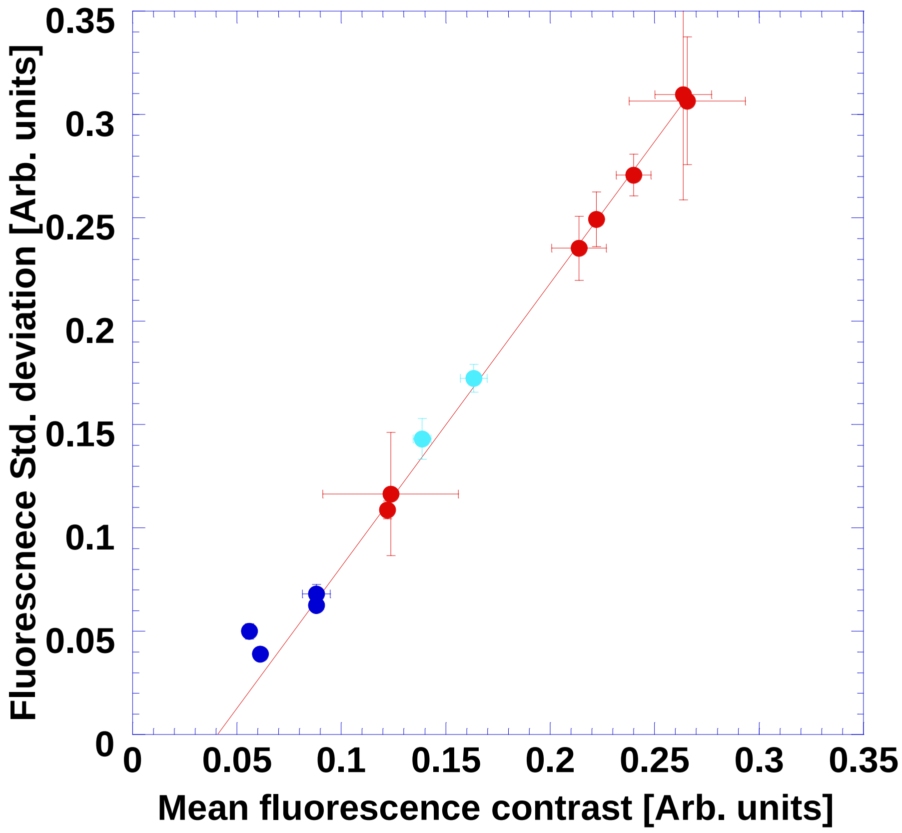
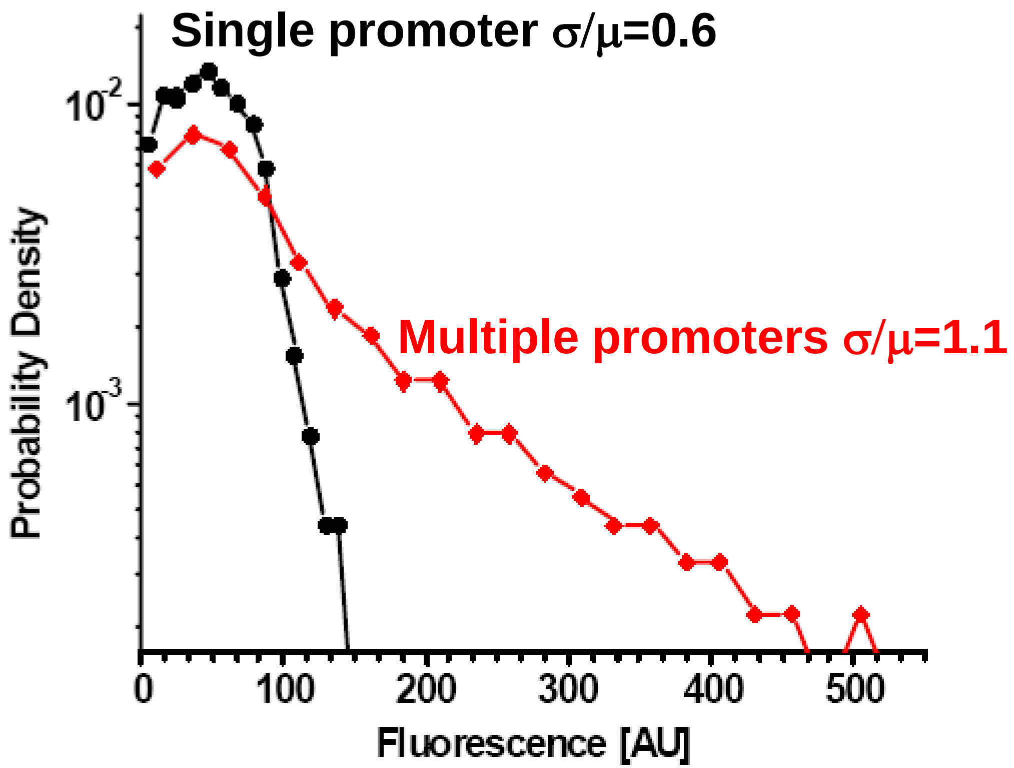
Chih Kuan Tung

Rockefeller: Albert Libchaber

Distribution characteristics
At steady state

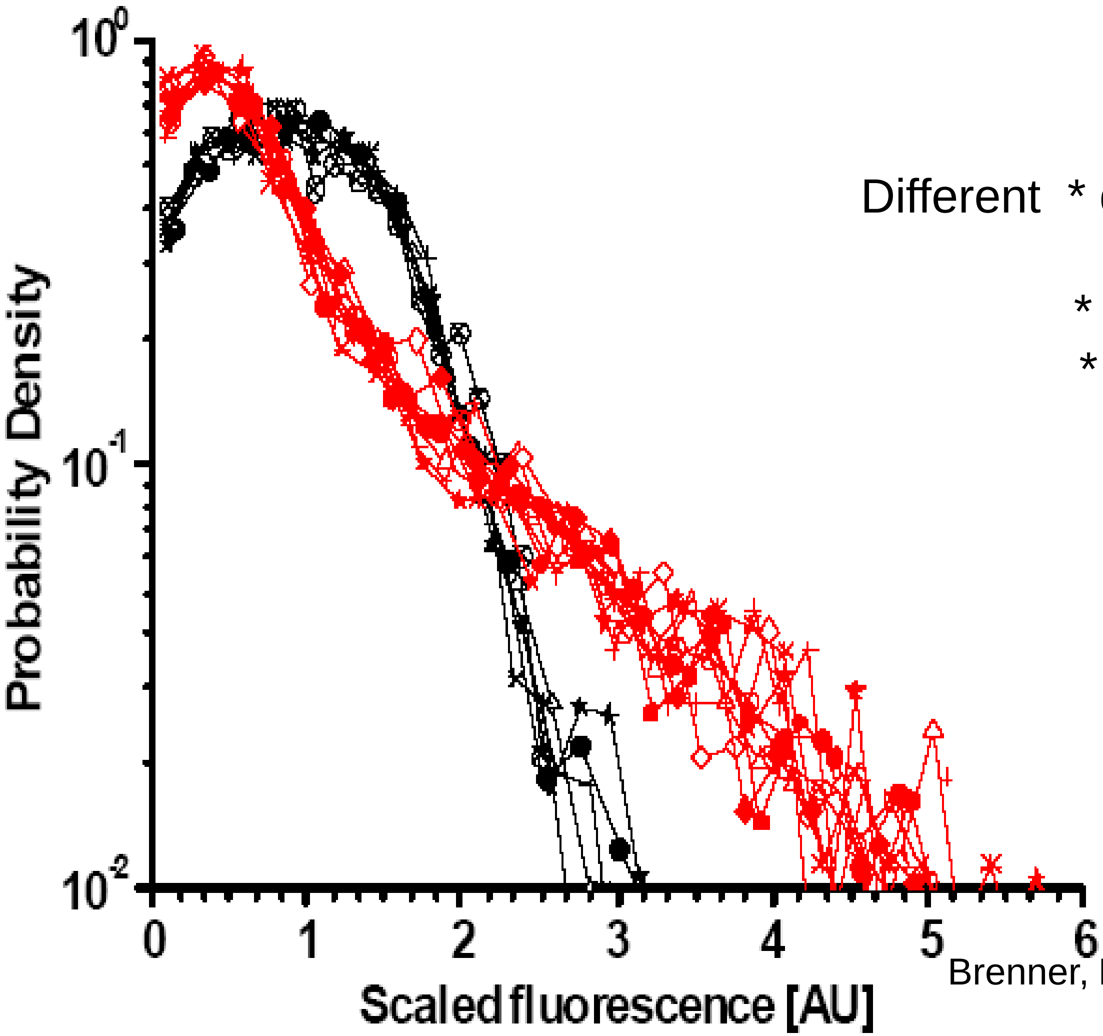


Exponential tails in expression distributions



Brenner, Farkash & Braun 2006

Two universality classes of steady-state distribution shape



Different * dilution rates
(-> growth rate)
* limiting nutrient
* culture density

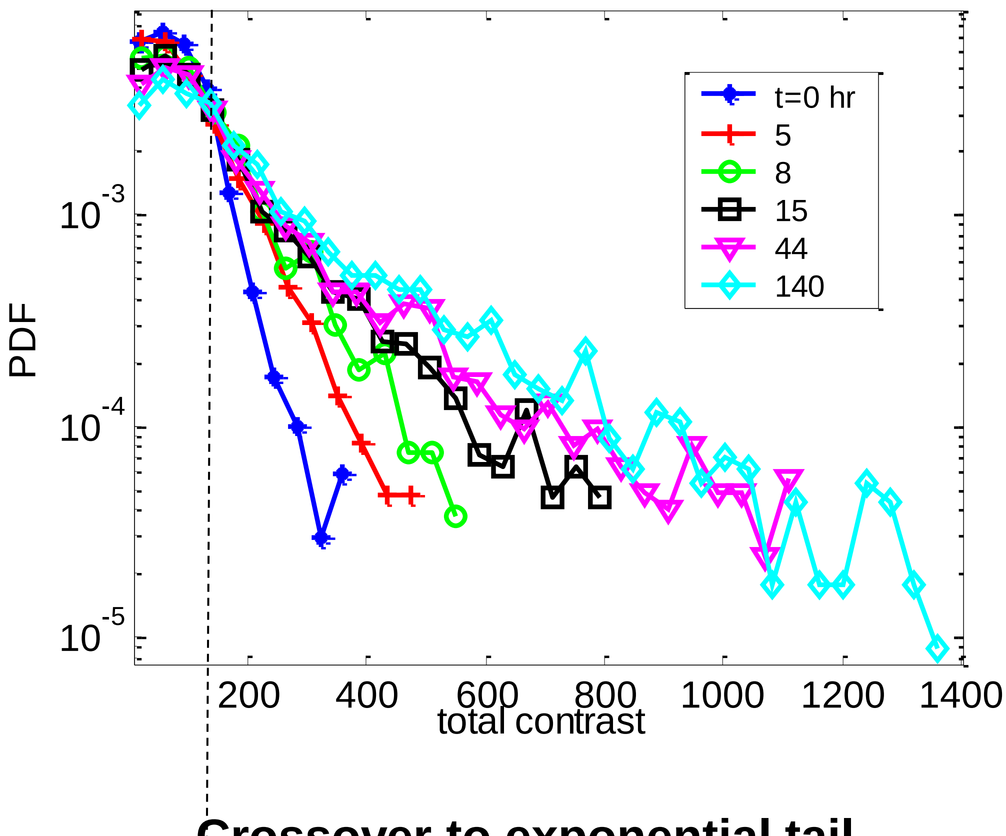
Brenner, Farkash & Braun 2006

<u>Time scales:</u>

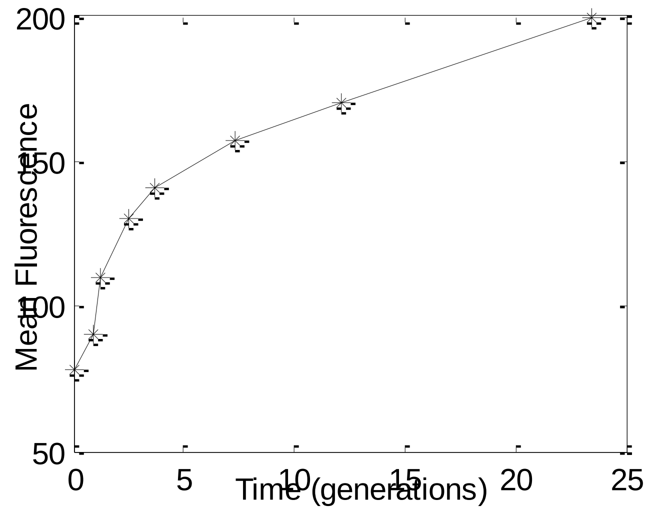
- Biochemical interactions ~ < sec
- Genetic regulation ~ sec - min
- Protein production & degradation ~ min
- Generation time ~ hr
- Population dynamics ~ several gen
- Mutation take-over time ~ 50 gen

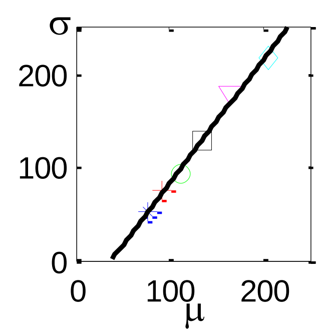
Gene induction – a population point of view

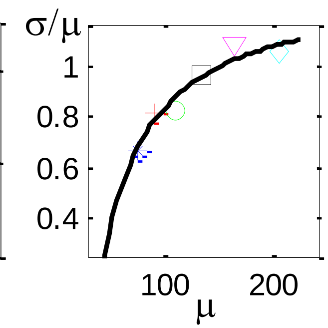
* Note: under an essential regulatory system! GAL in pure galactose



Crossover to exponential tail

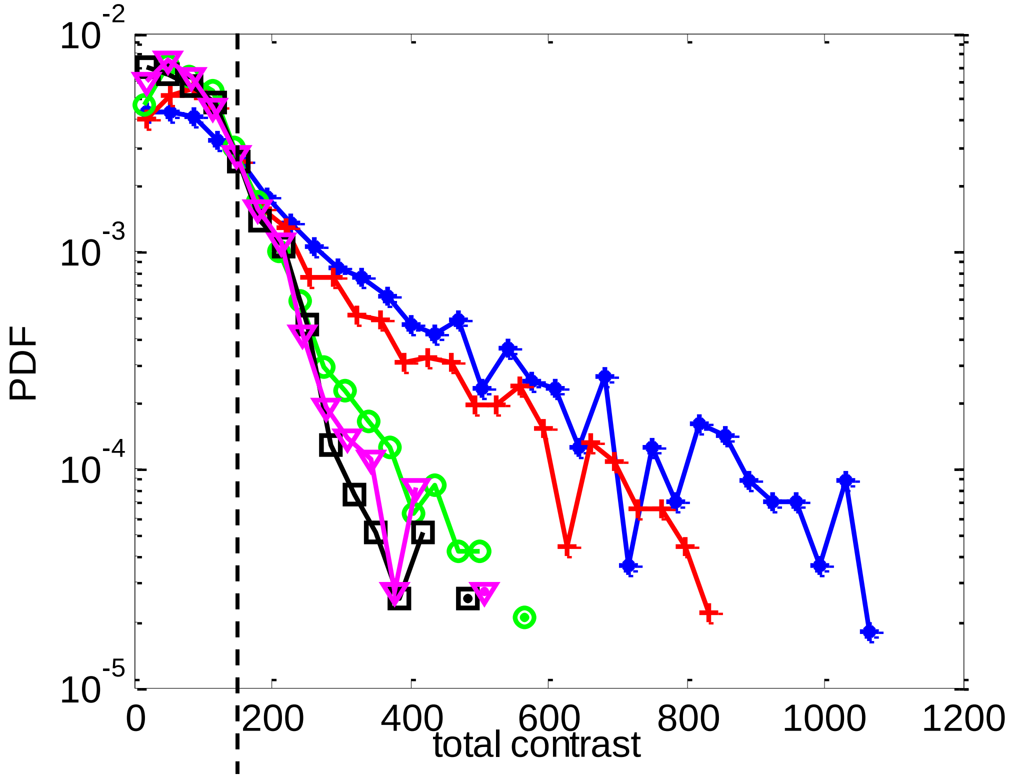


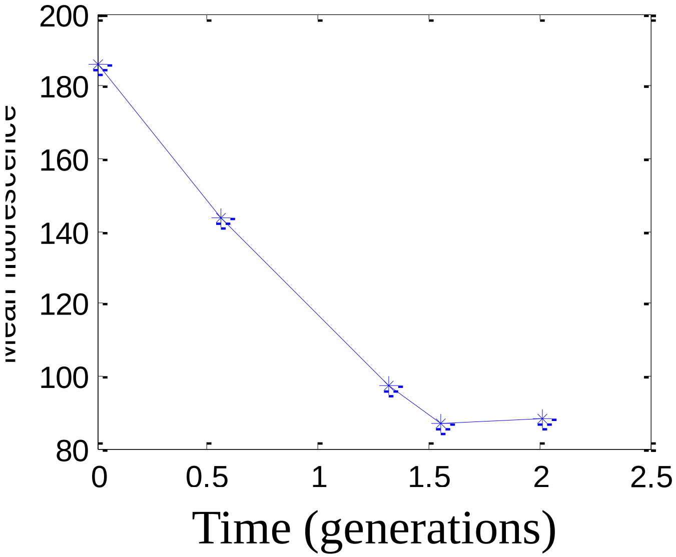


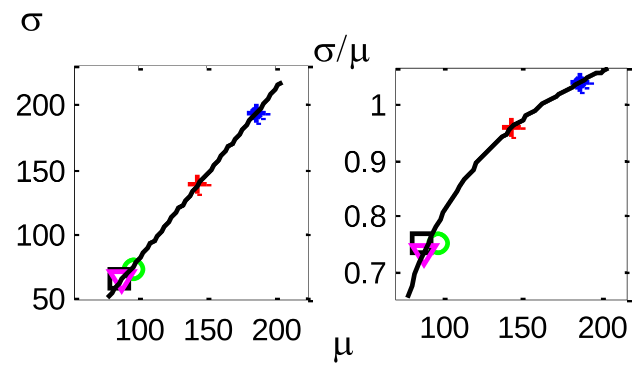


Induction at t=0. Long time scale

Gene repression – a population point of view

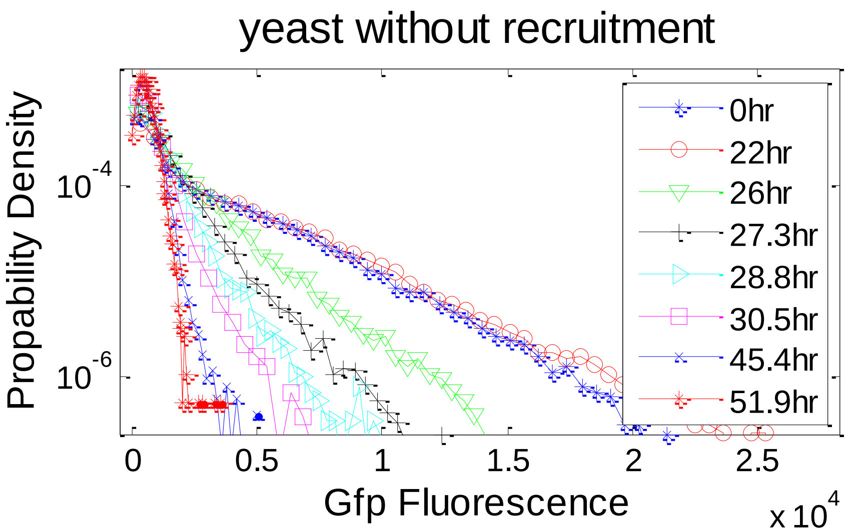


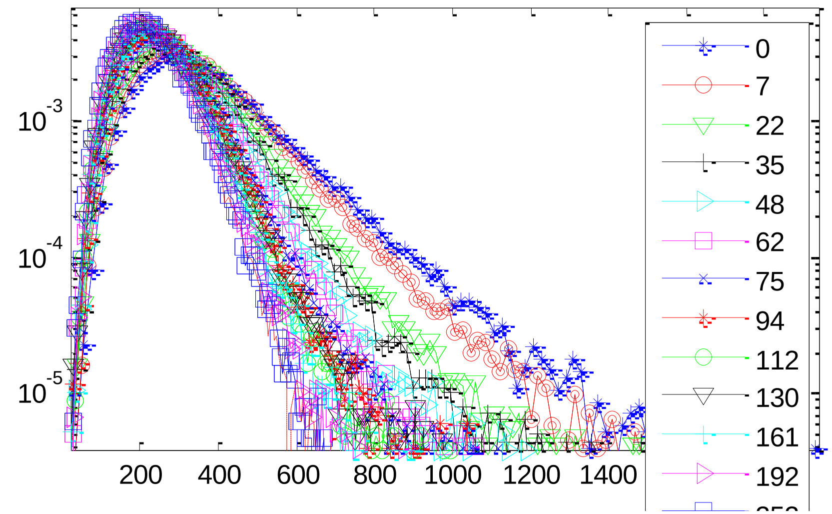




Induction at t=0. ~ 1 generation time scale

Results of comparative experiments: Distributions



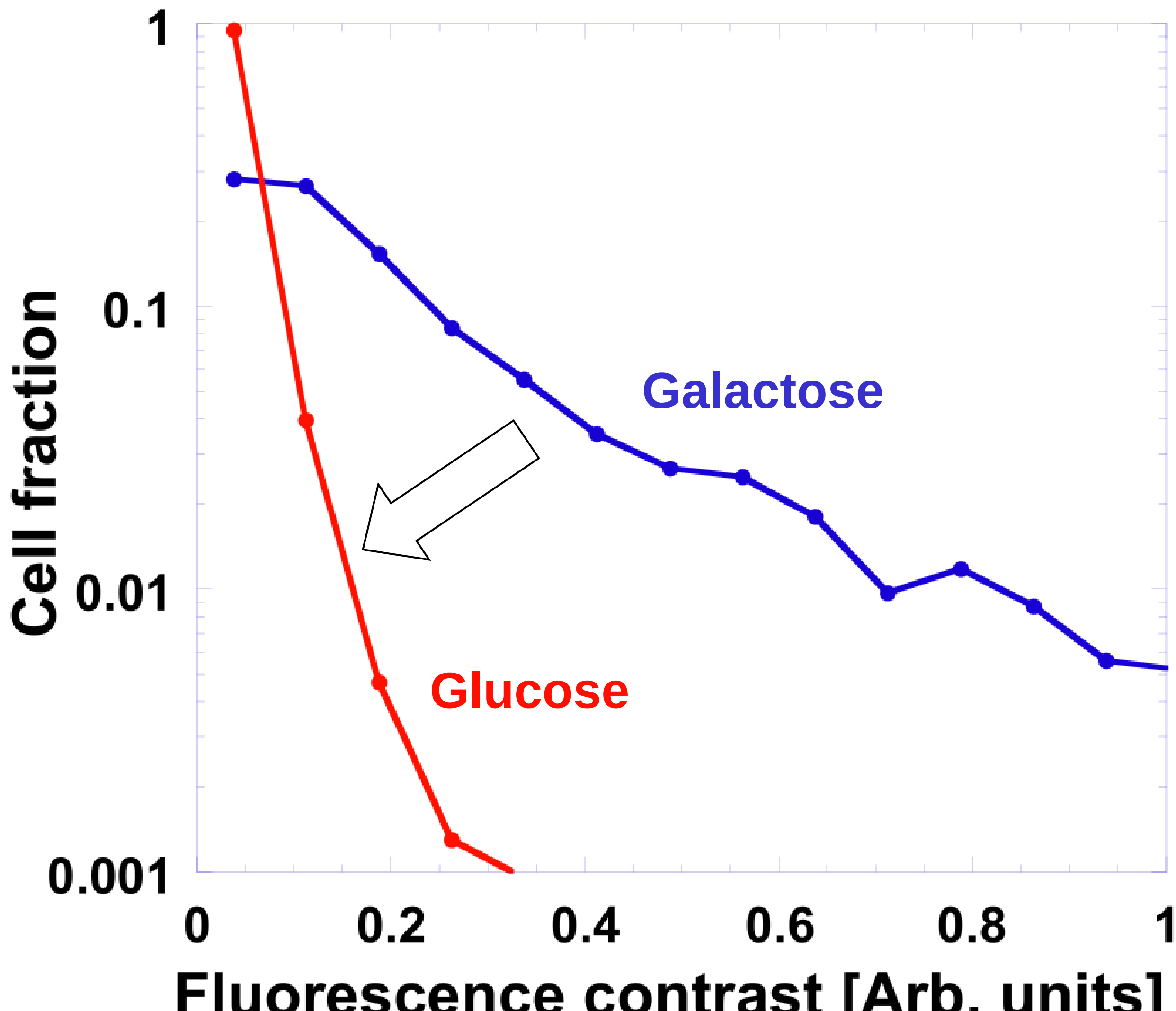


Shapes of distributions along time:

* An exponential tail is retained through dynamics

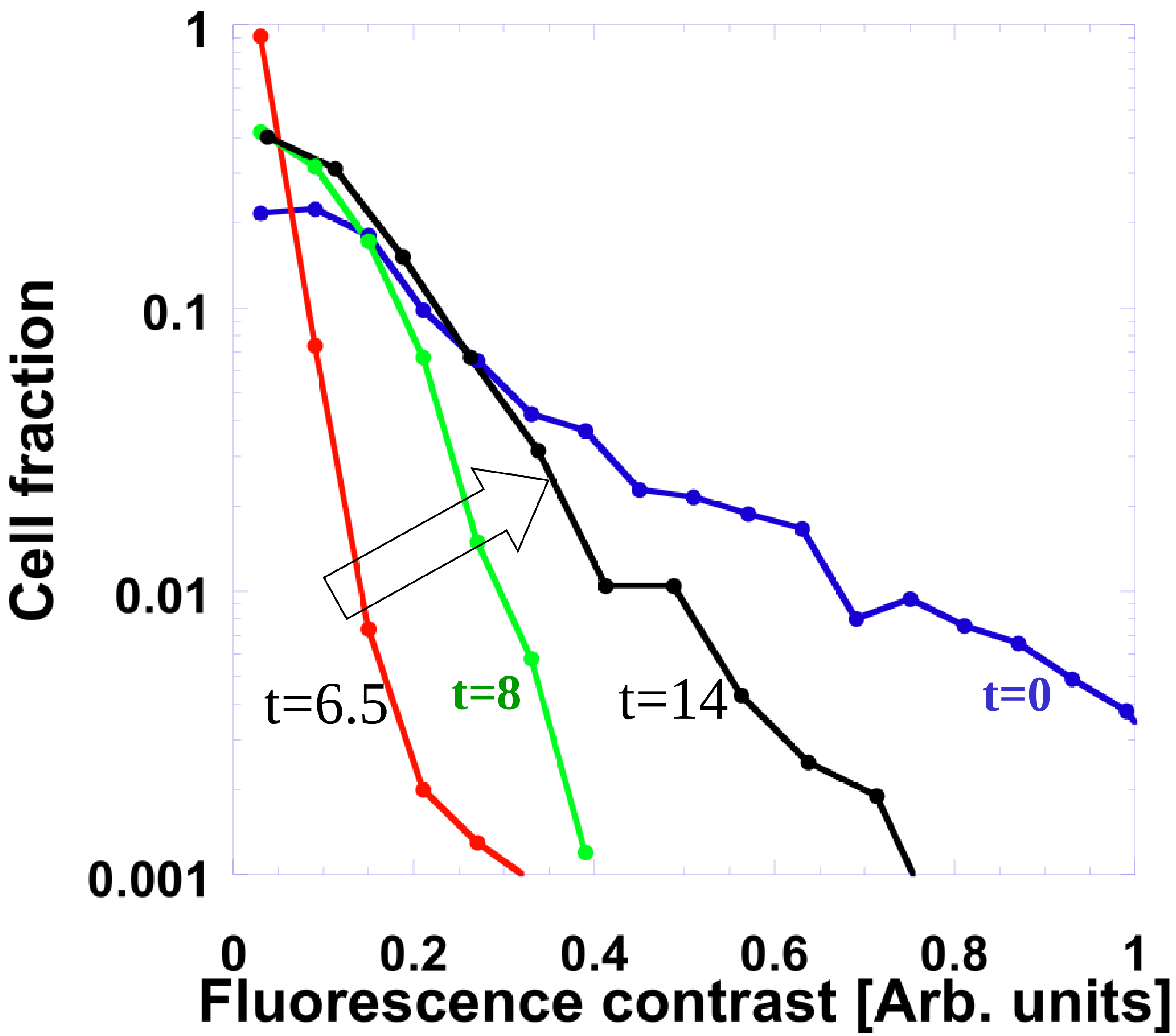
* Yeast have higher copy number - different head shape

Over longer times: glucose repression…



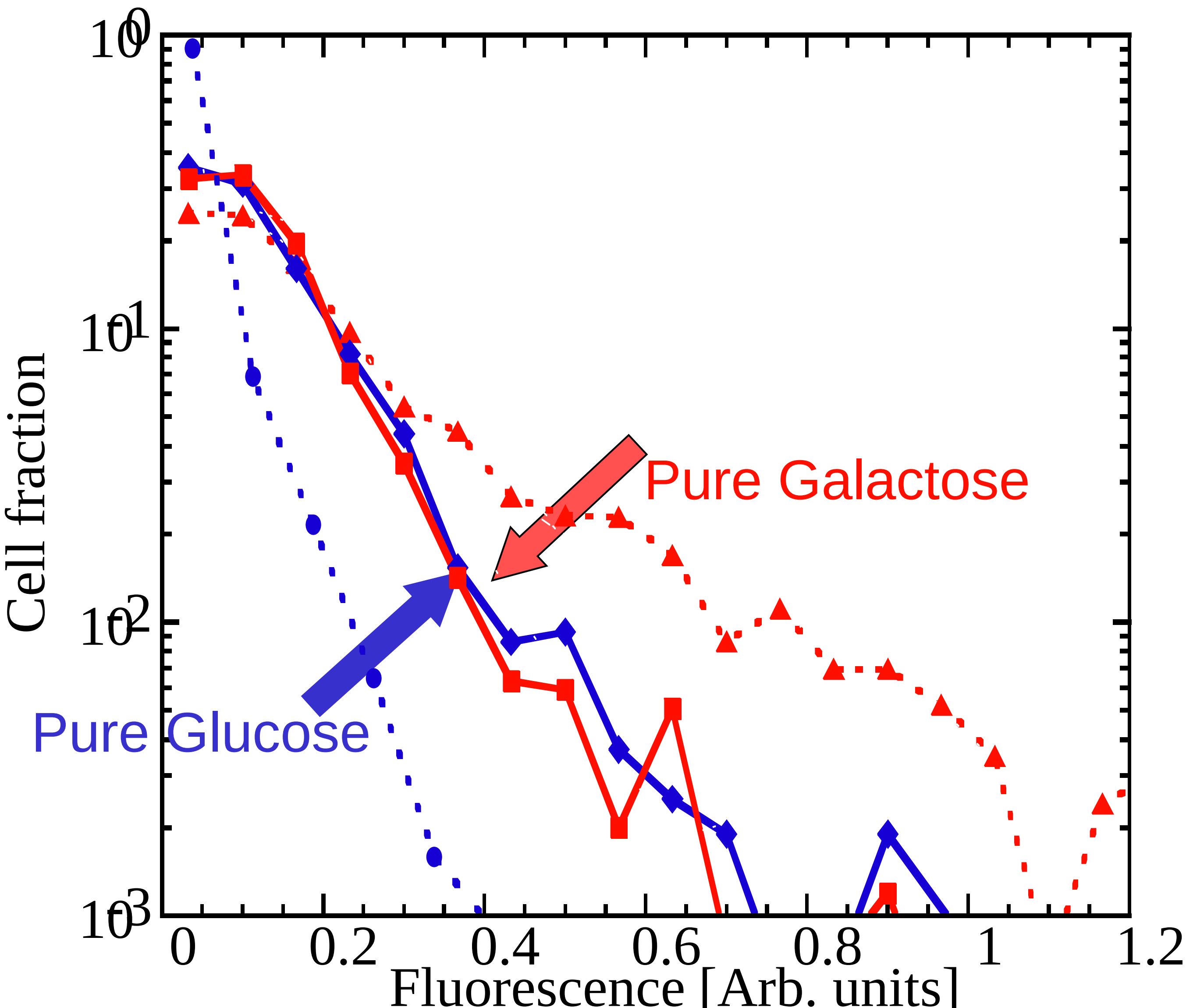
Switch medium
From 2% gal
To 2% glu

…and adaptation



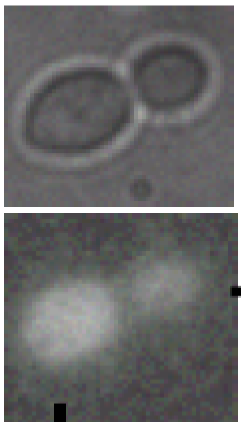
Time in hrs after switching from galactose to glucose

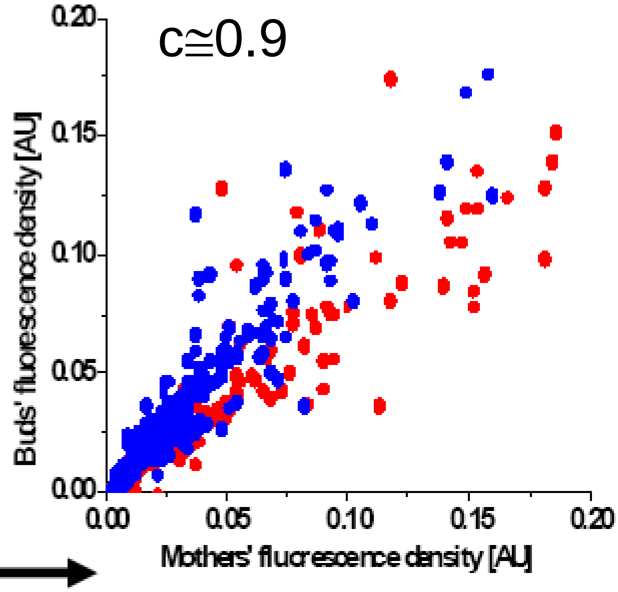
Invariant steady-state



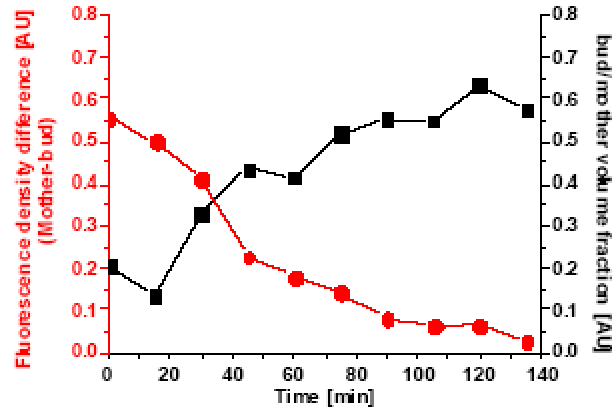
(Braun & Brenner, J. Phys. Biol. 2004)

Protein correlations among generations in the budding yeast





Real-time microscopy



Statistics in a
Steady-state population

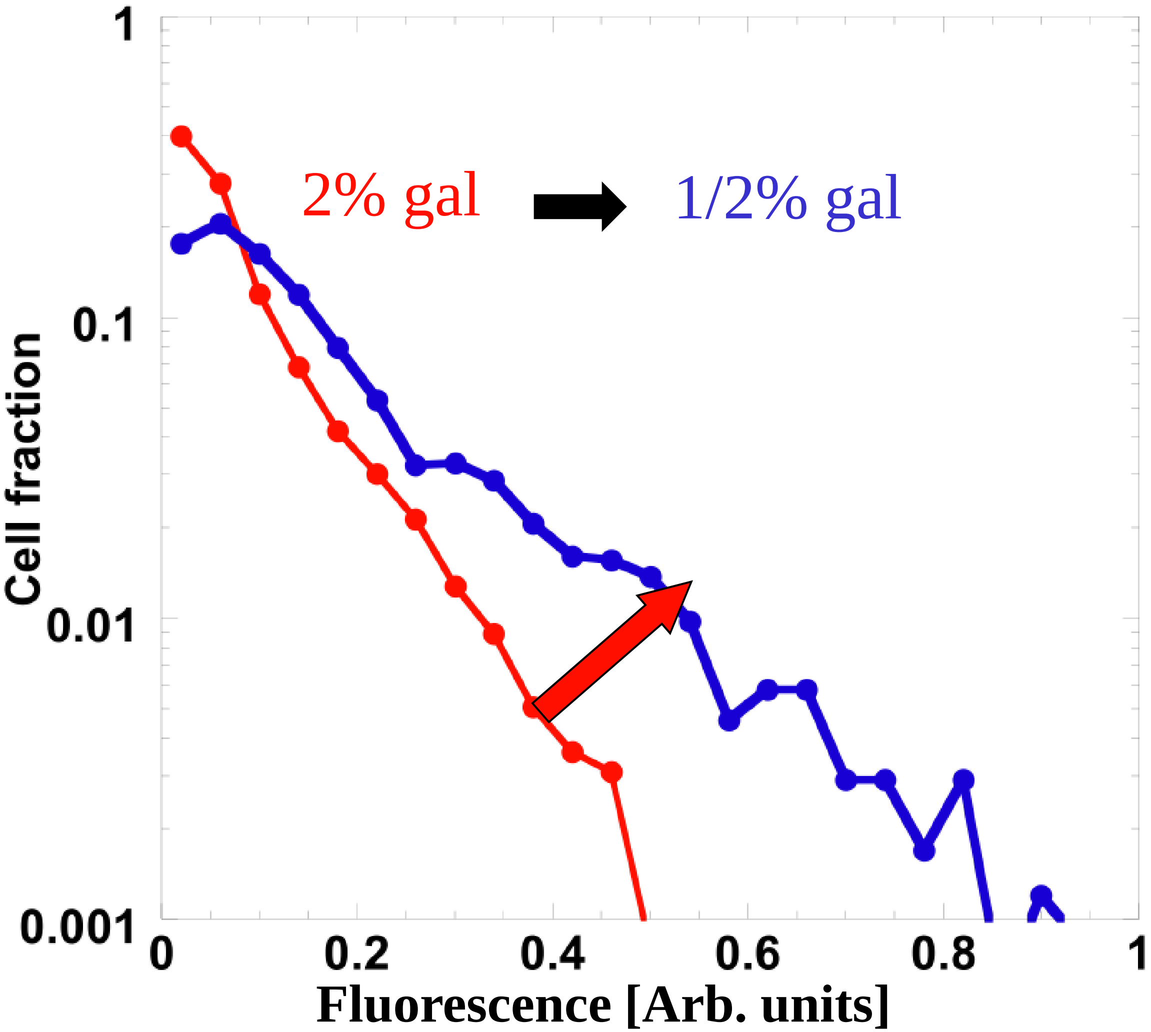
Conclusion – strong dissipation by fractional loss to next generation

Conclusion – a population is not an ensemble
of independent particles,
but a dynamical system!

The steady state distribution cannot be dominated by internal cellular noise, but by a balance of population forces…

These are: a production source and dissipation by dilution

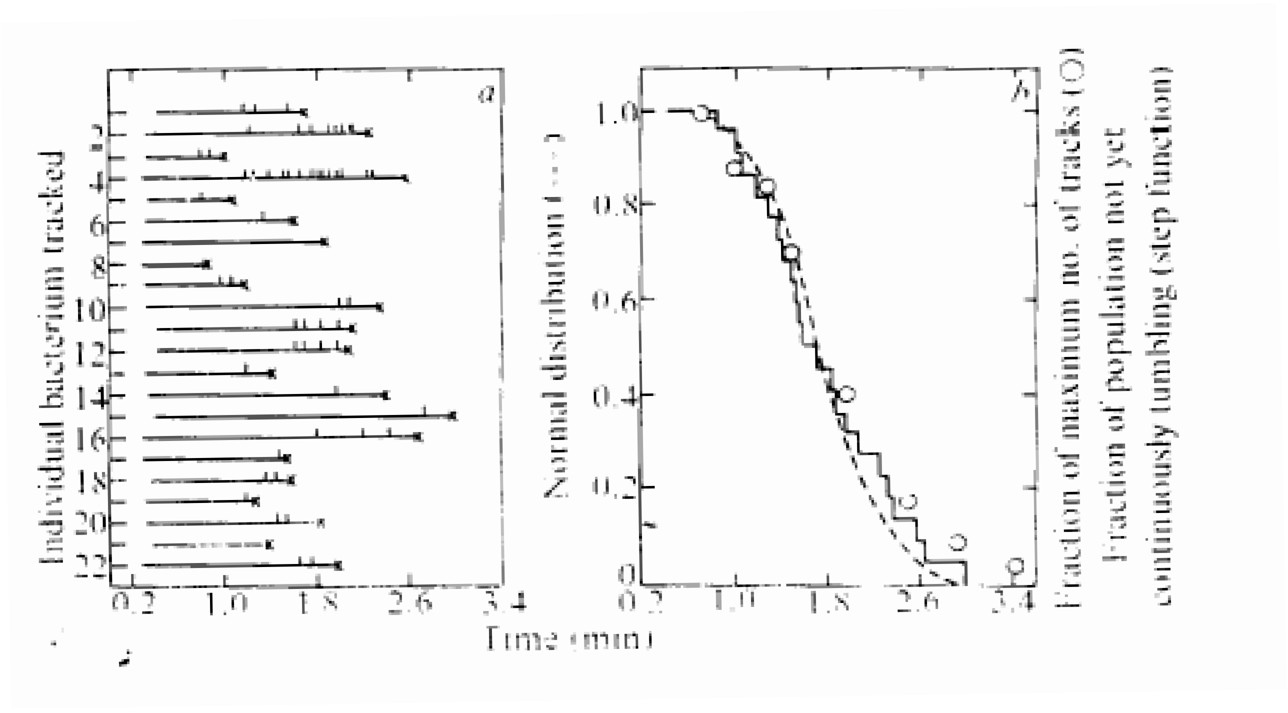
Transition stimulated by pressure in galactose



Note: pressure increases protein variation!

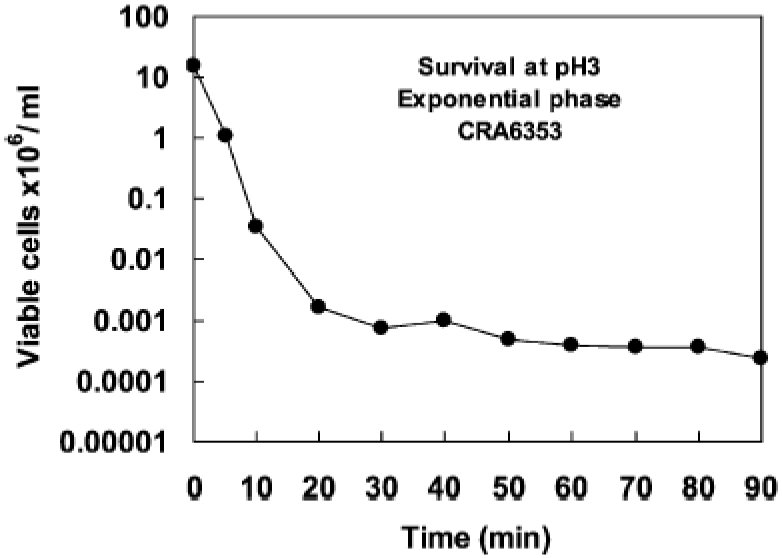
Phenotypic (non-genetic) variation

Chemotaxis relaxation time in isogenic bacterial population

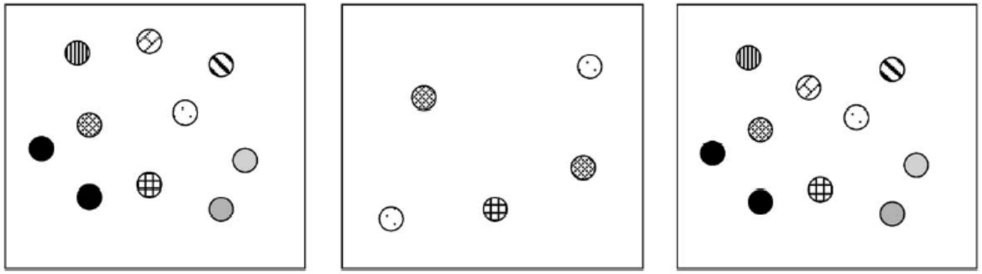


(Spudlich & Koshland 1976)

Survival by "transient adaptation"



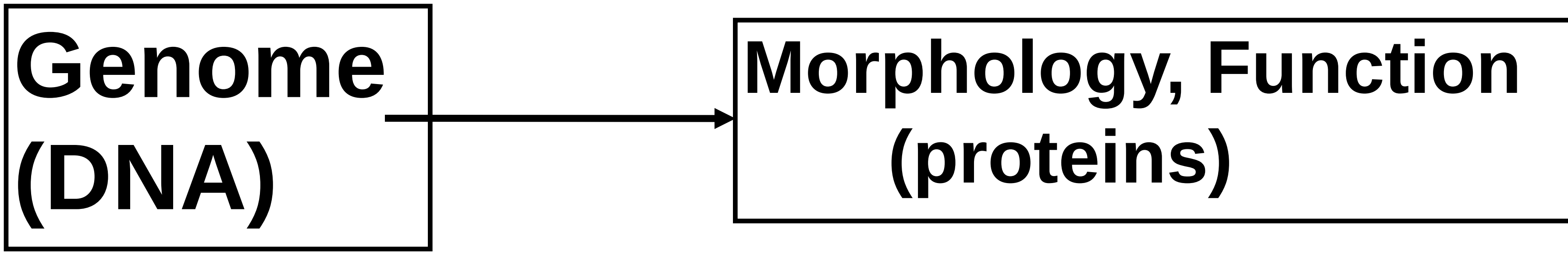
Jordan et al. (1999)



Booth (2002)

A fraction of the population survives and proliferates after the stress is over

Genotype -> Phenotype transformation



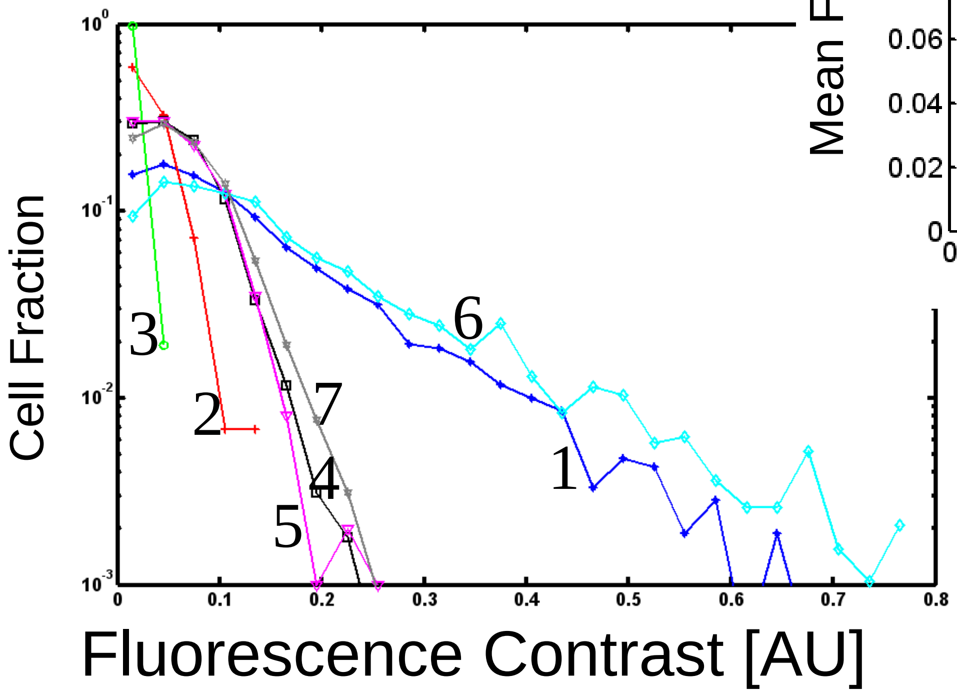
“genotype” “phenotype”

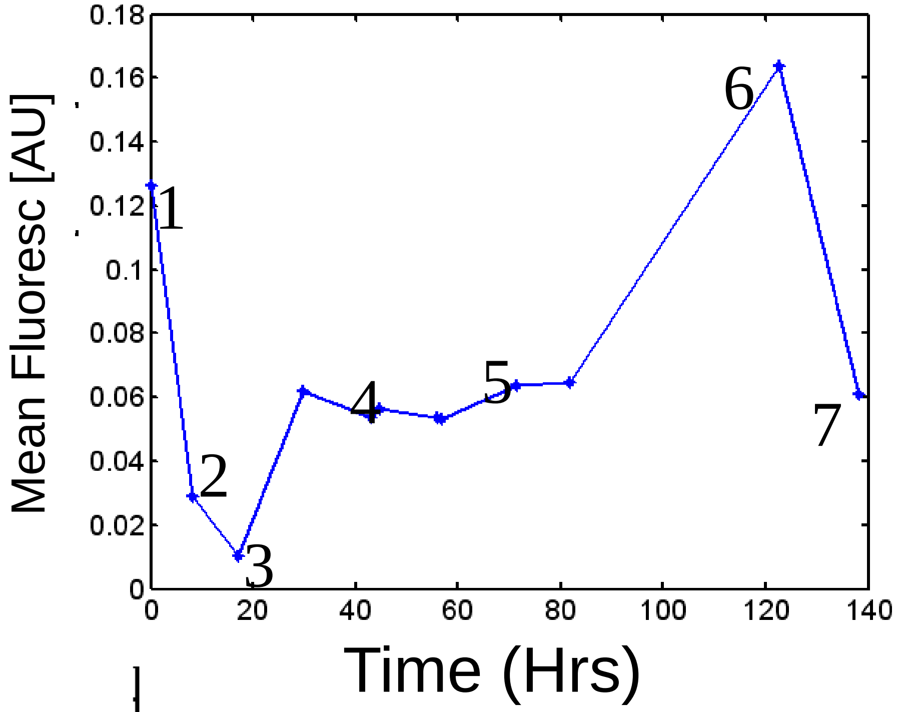
This transformation is complex, nonlinear,
degenerate, multi-valued

* selection processes act on the phenotype!

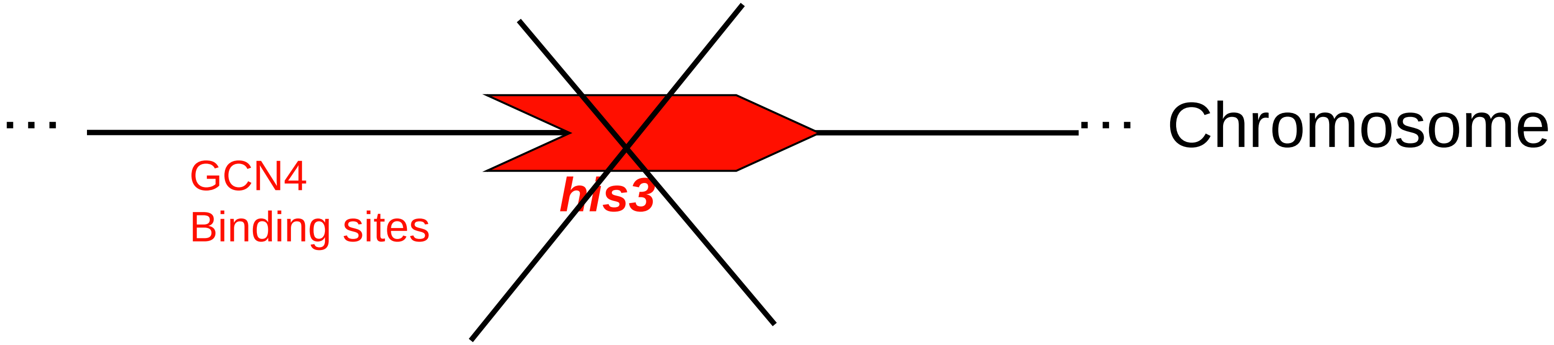
Memory in the population

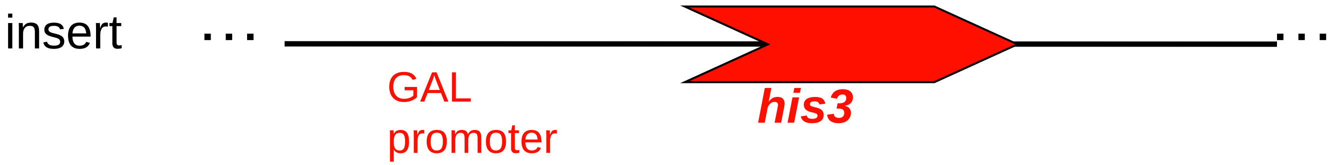
(Batch experiment)





Synthetic Gene Recruitment



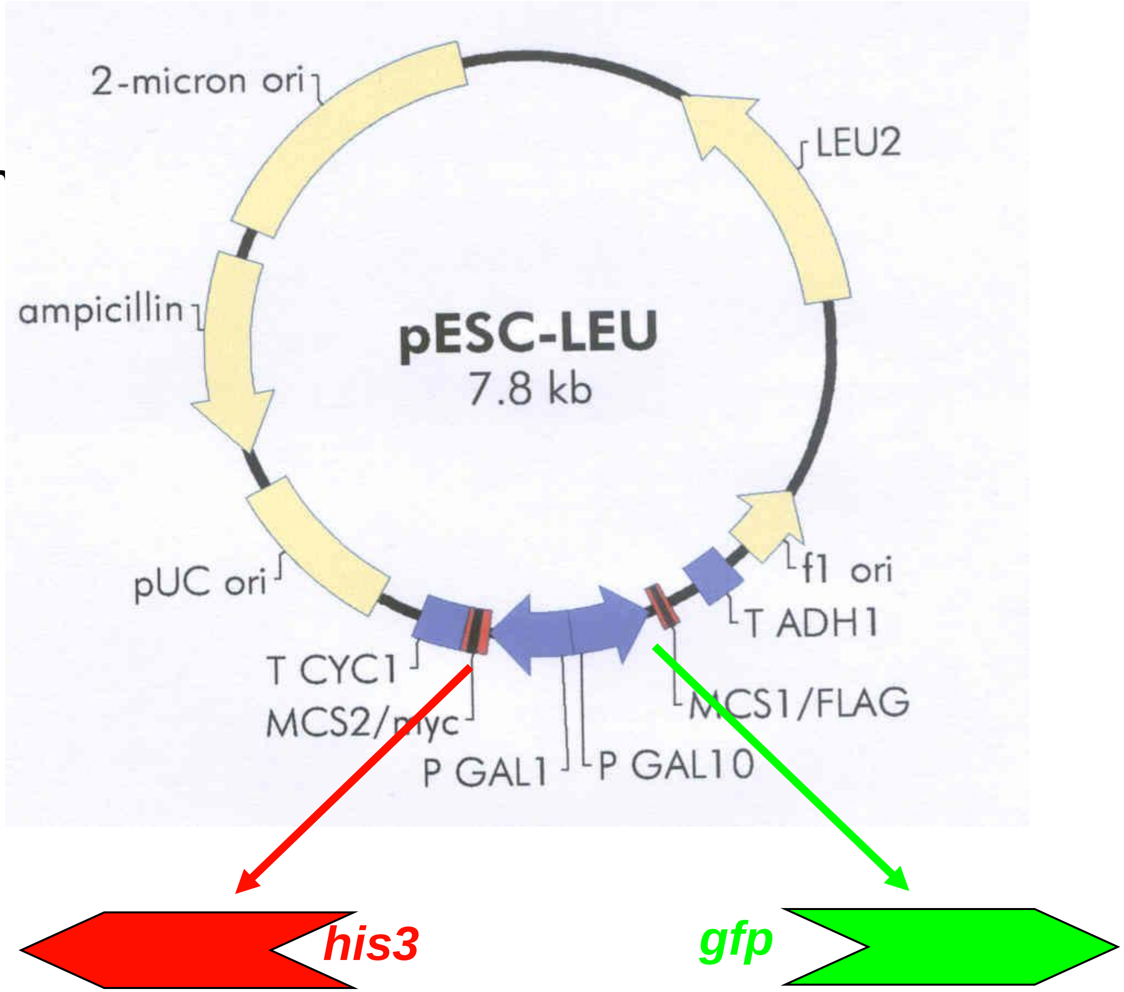


* HIS3 gene recruited under the GAL regulation network!

induce by galactose, repress by glucose…

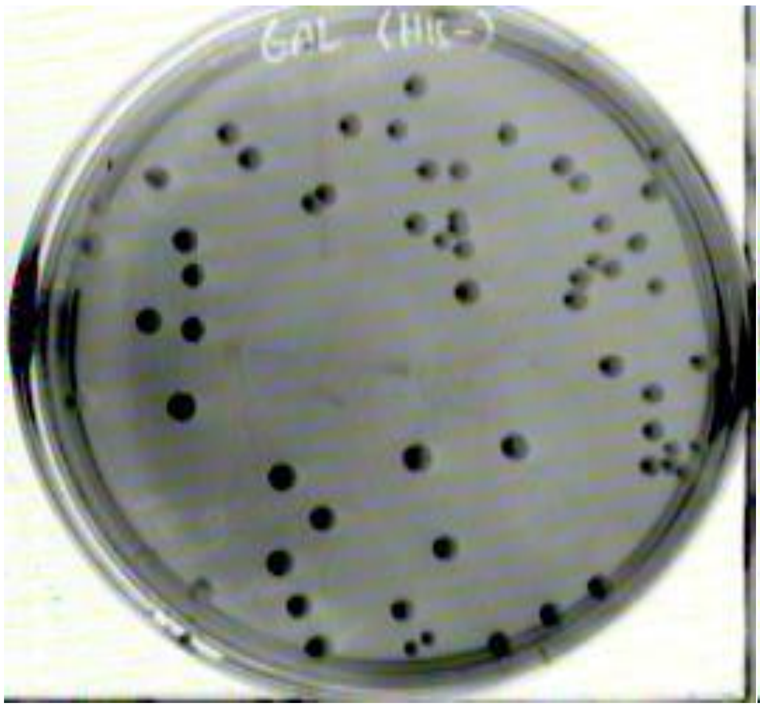
Synthetic Gene Recruitment

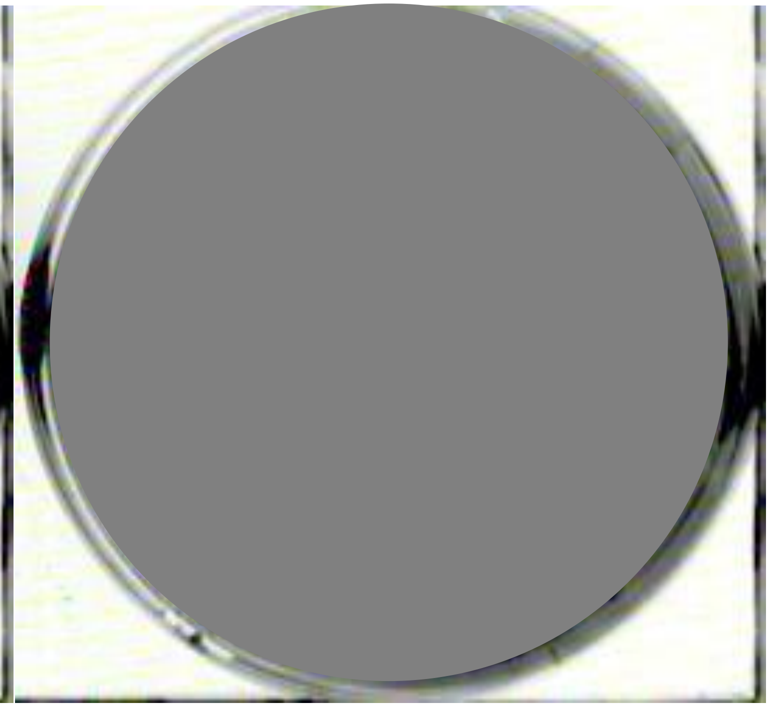
Grow in (his-) mediur
add 3AT



Checking for functionality of recruited gene:

Colonies 2 days after plating,





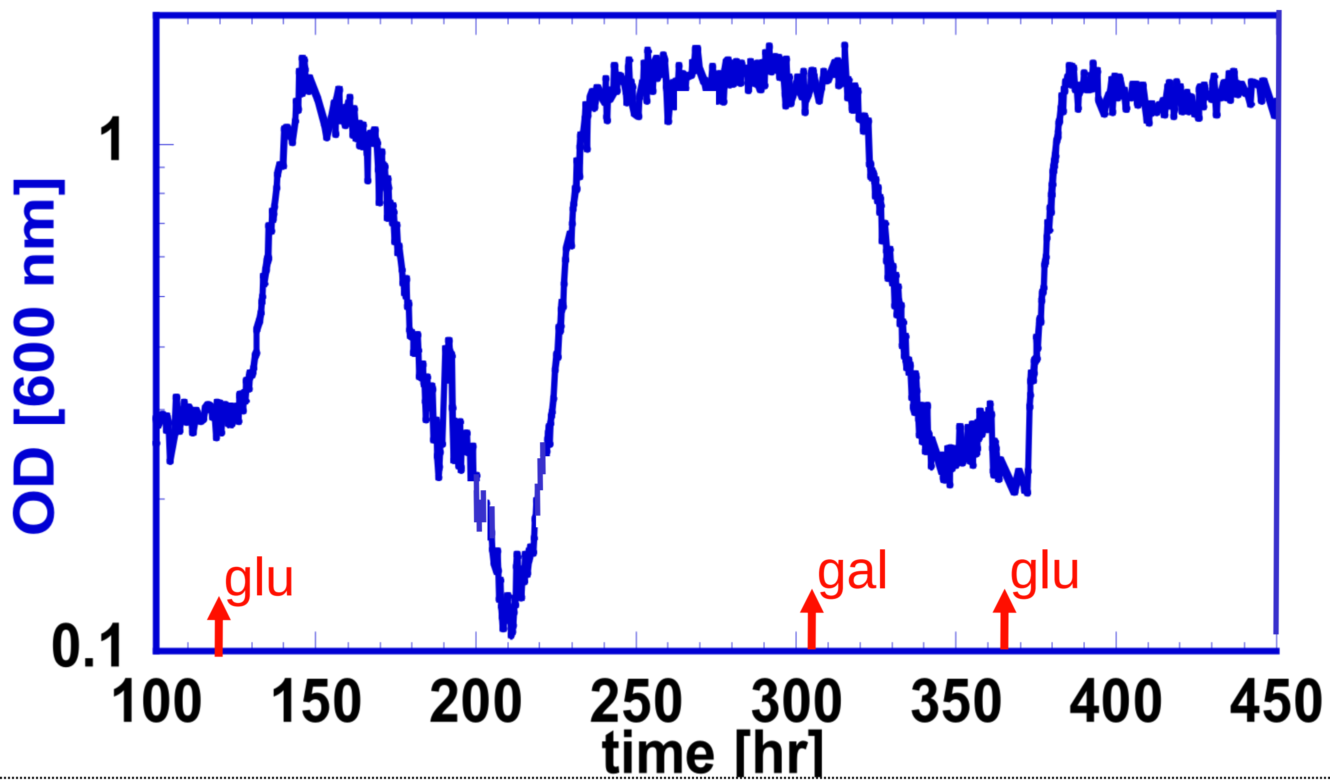
Normal growth on galactose

No growth on glucose

Long-term dynamics used for survival

Gal-Glu transition with gene recruitment perturbation

On-line measurement system



10 generations

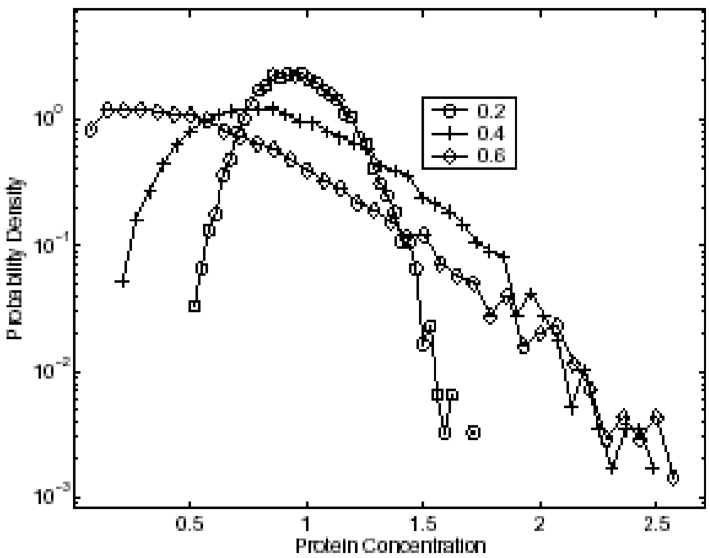
Summary of experimental results

- Large variations even in a regulated protein. Exponential distributions with large std/mean ratio.

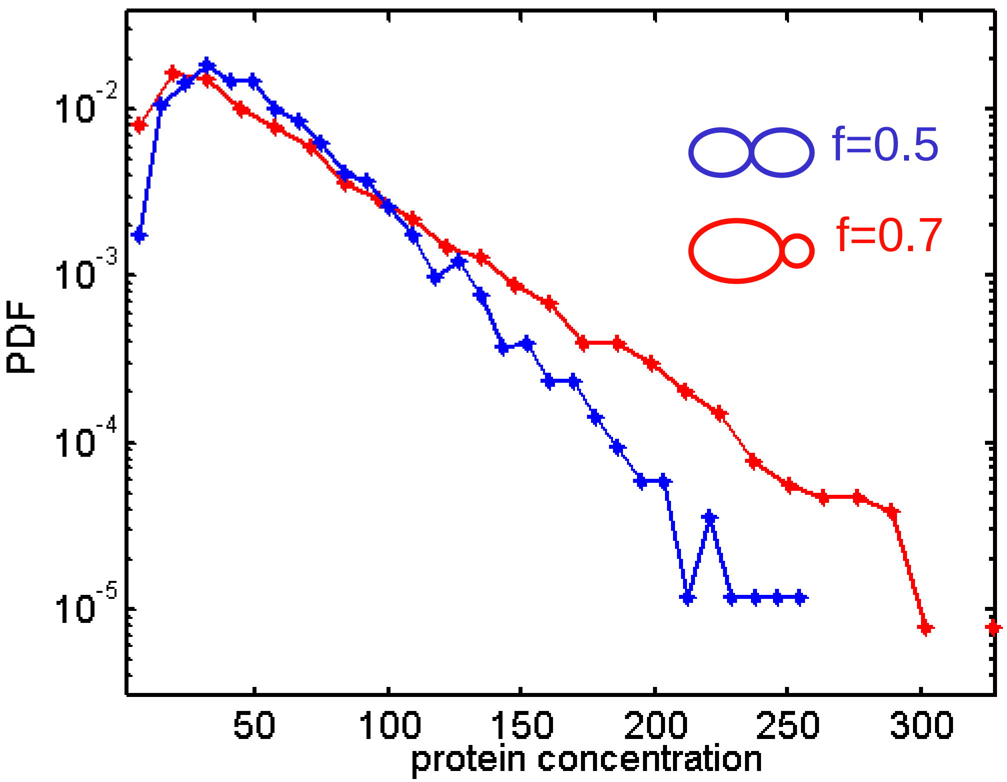
- A population is not an ensemble of independent particles… Variation maintains correlations over times > generation A marked asymmetry is observed between two directions of relaxation.

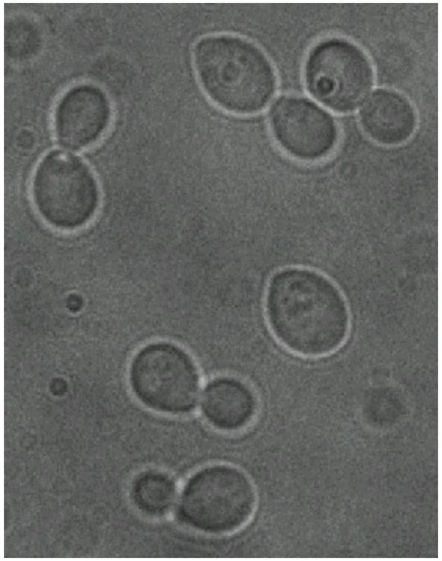
- Given a variable source of protein production, the distribution is insensitive to the details of this source. The distribution is shaped by two opposing forces – a source and a dissipation force.

Effects of “noise” in division

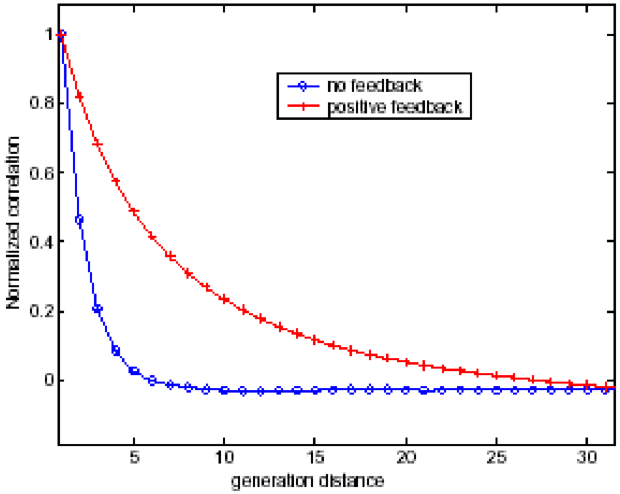


Effects of asymmetric division



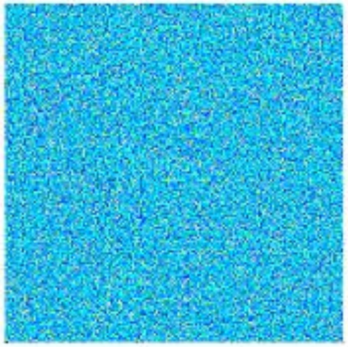


Effects of feedback in protein production



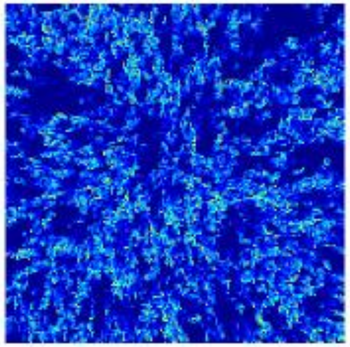
Protein correlations among generations

Growth in a "dish"



(a)

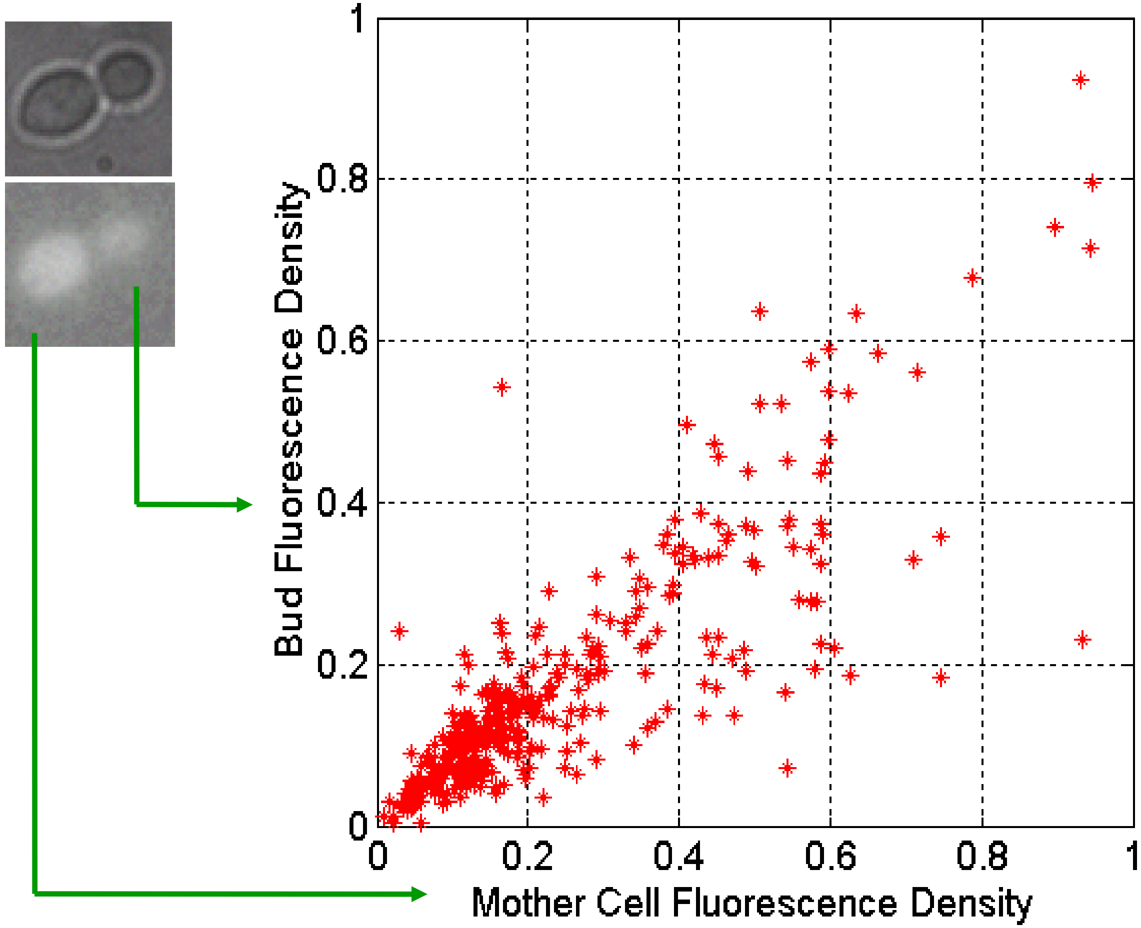
no feedback

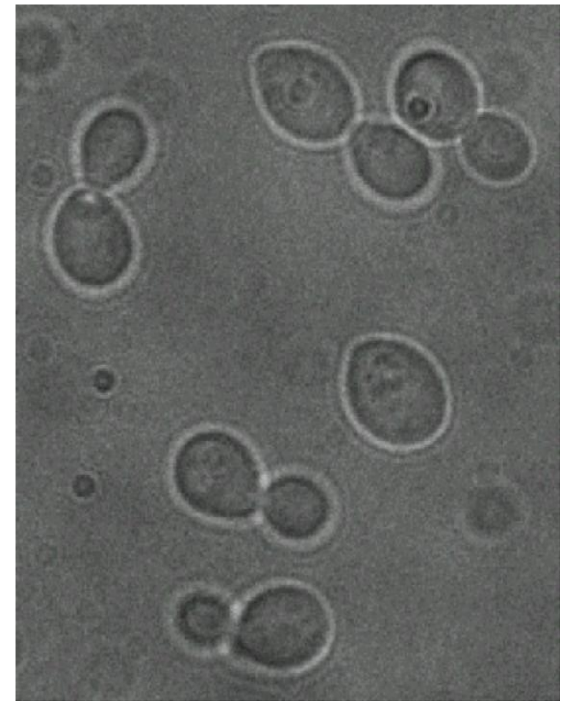


(b)

positive feedback

Protein inheritance



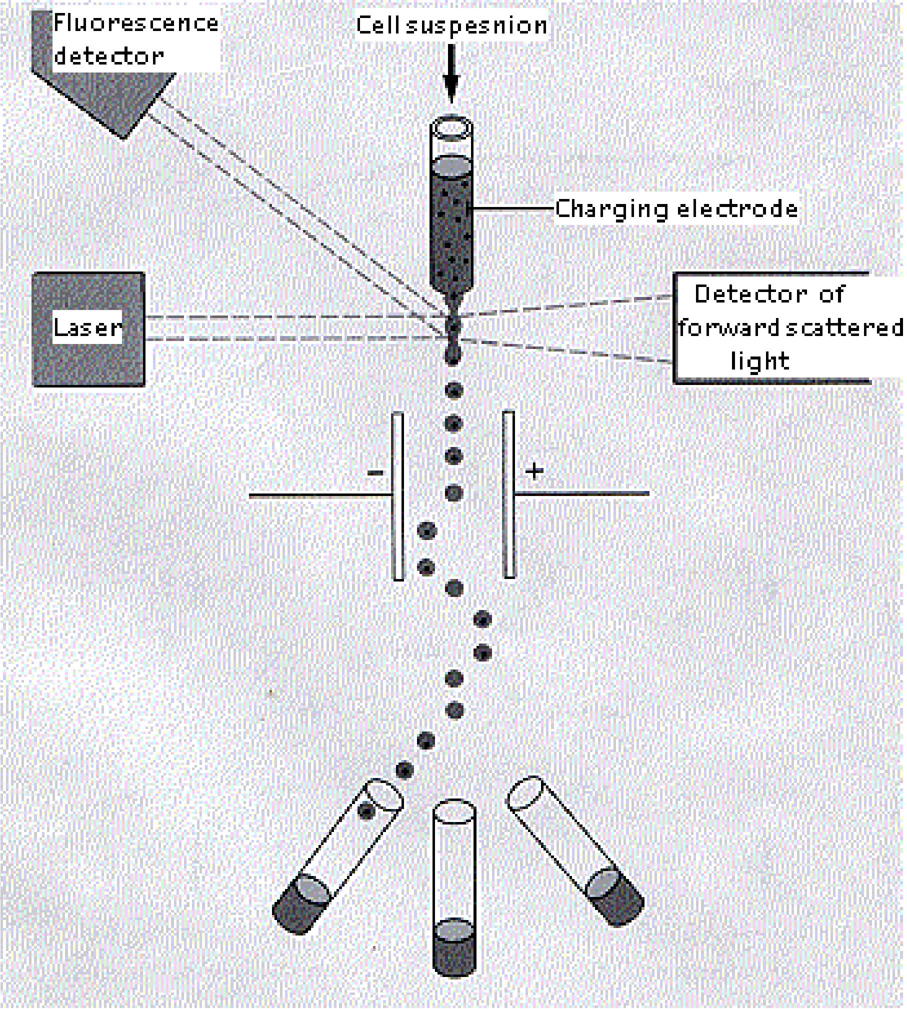


Idenifying
Cell families

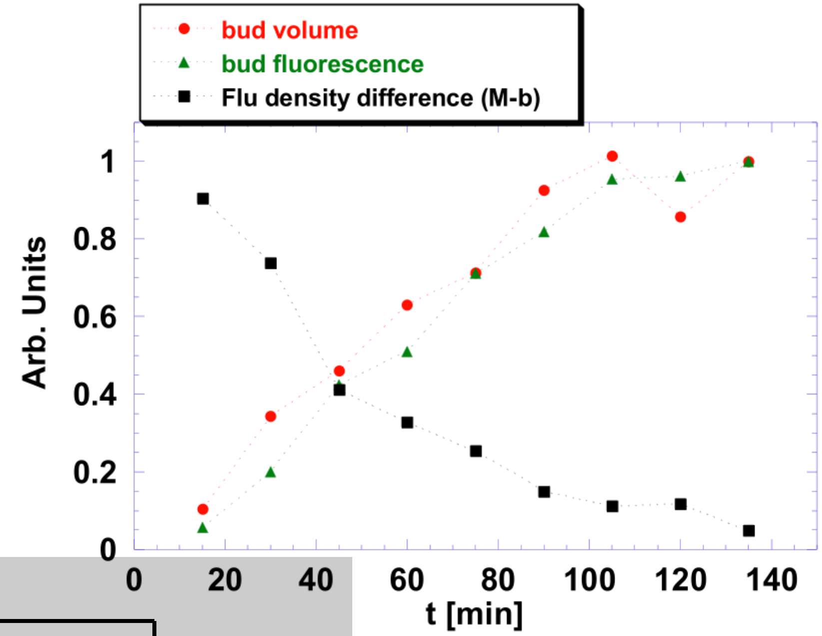
Time scales:

- Biochemical interactions ~ < sec
- Genetic regulation ~ sec - min
- Protein production & degradation ~ min
- Generation time (~ hr)
- Population dynamics ~ several gen
- Mutation take-over time ~ 50 gen

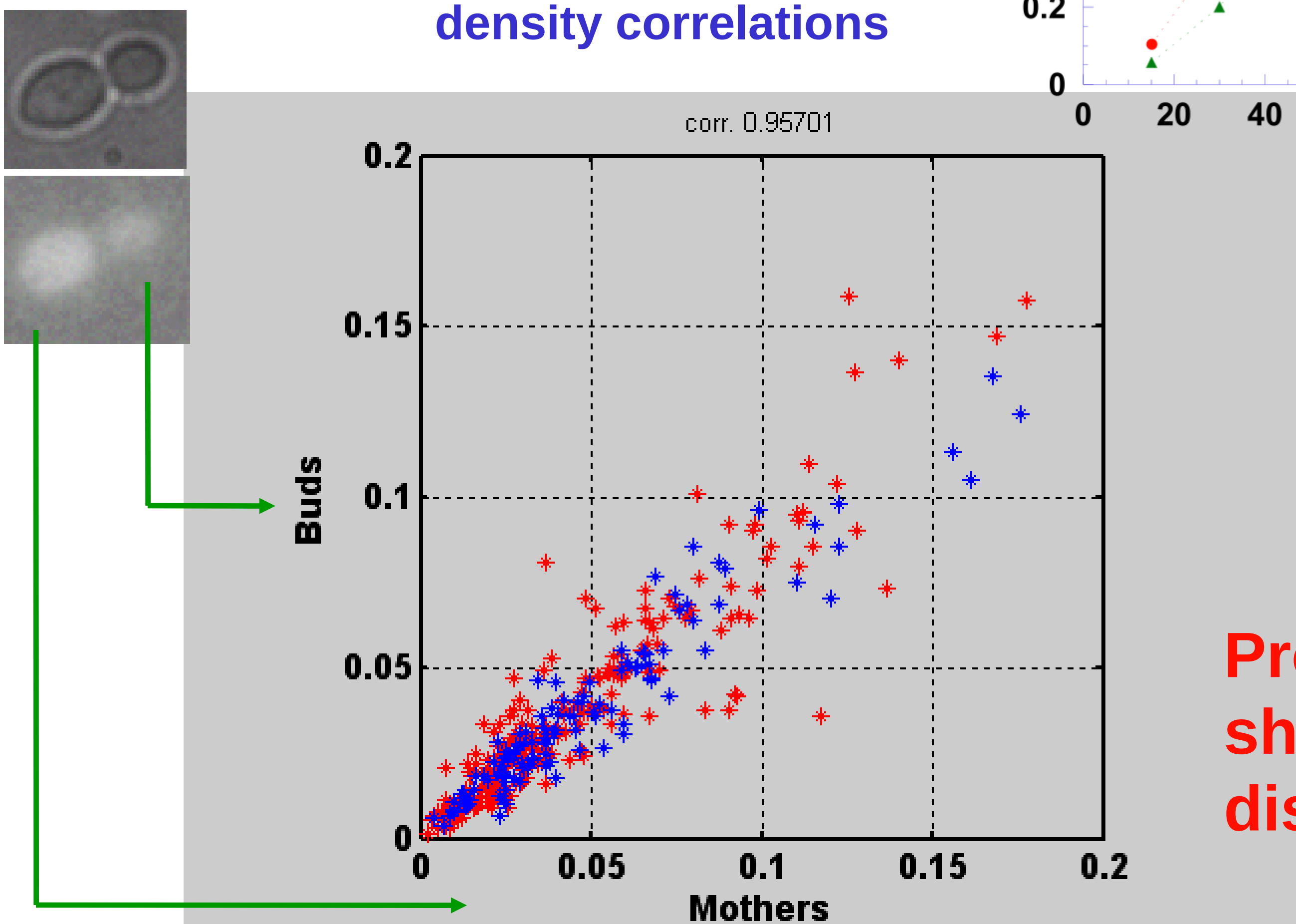
Fluorescence activated cell sorter (FACS)



Single-cell growth curves

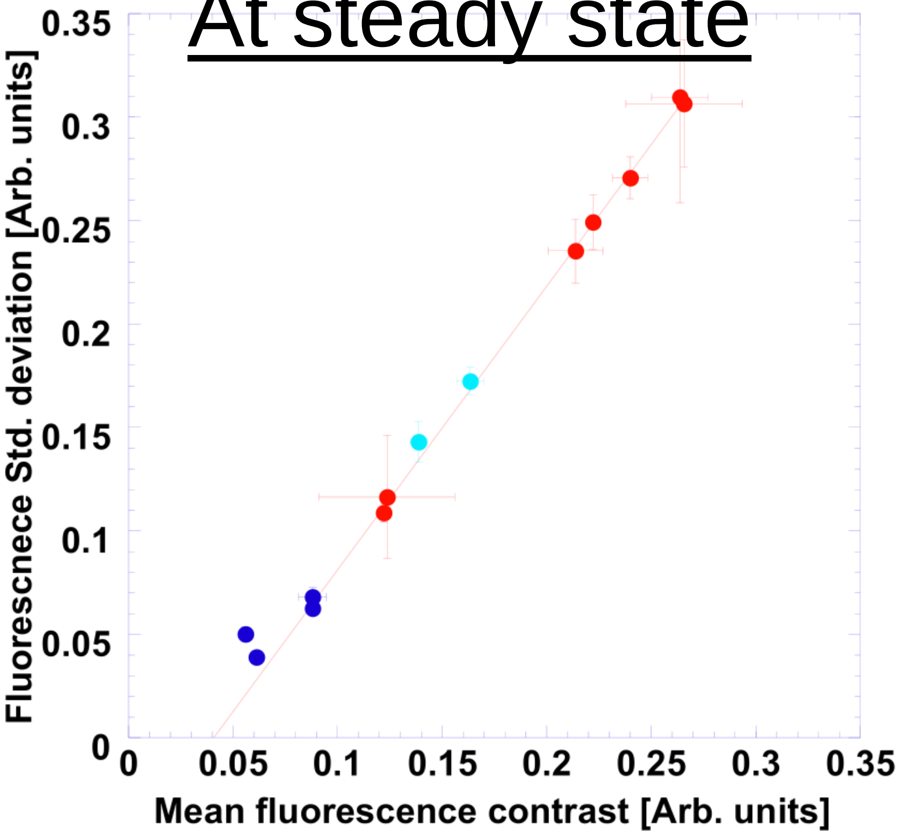


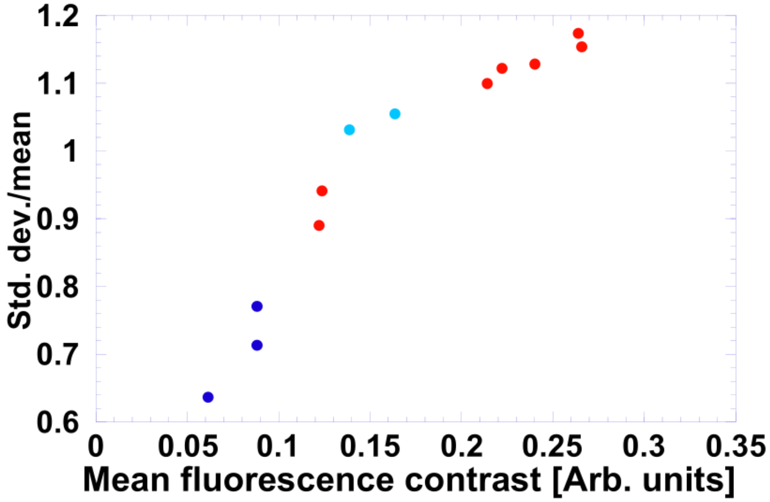
Mothers-buds density correlations



Protein inheritance shaping population distribution.

Distribution characteristics At steady state

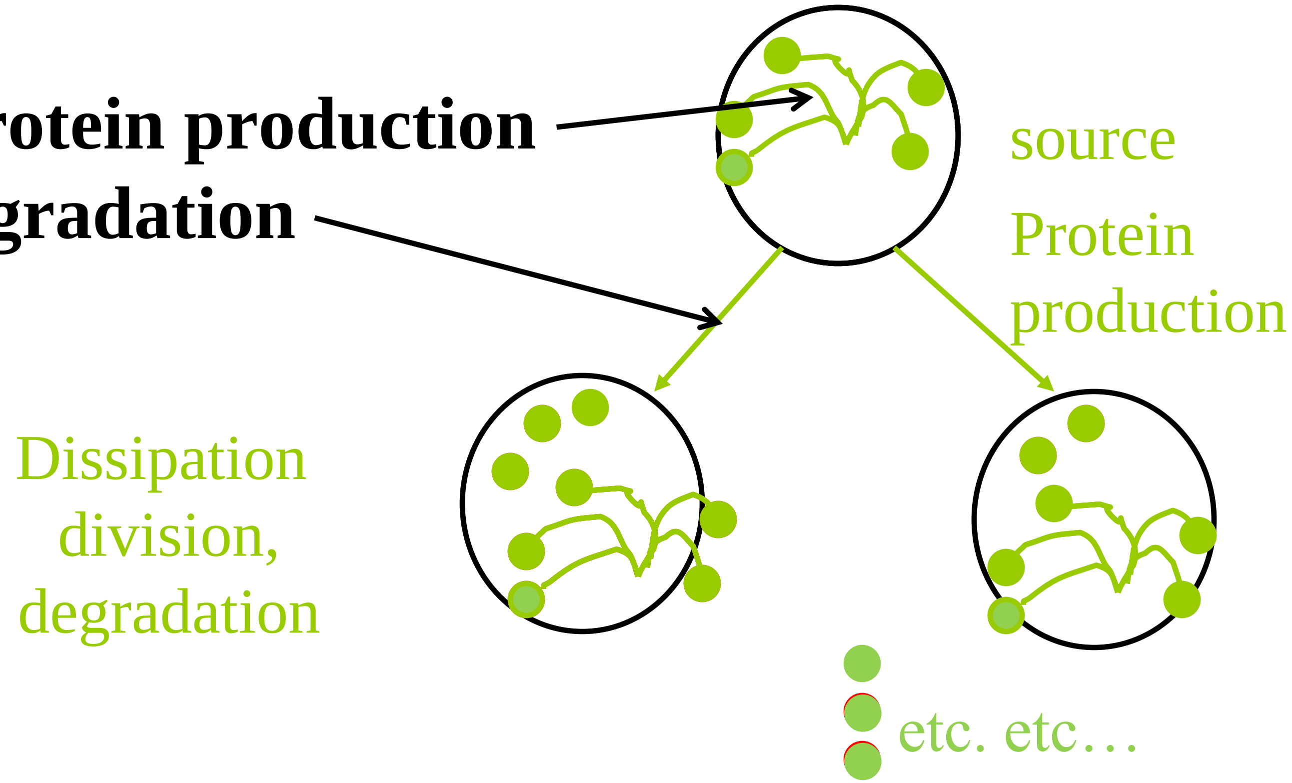




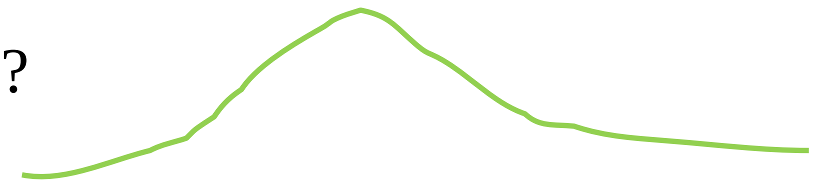
Underlying stochastic processes:

Stochastic protein production

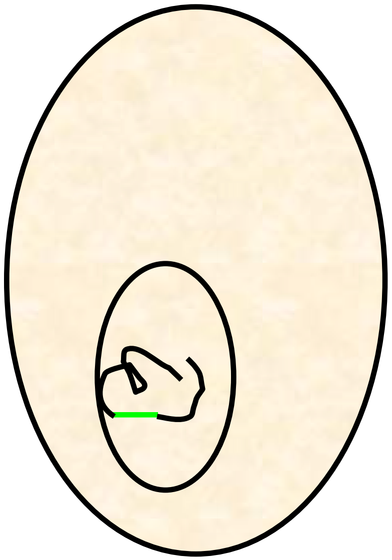
Division / degradation



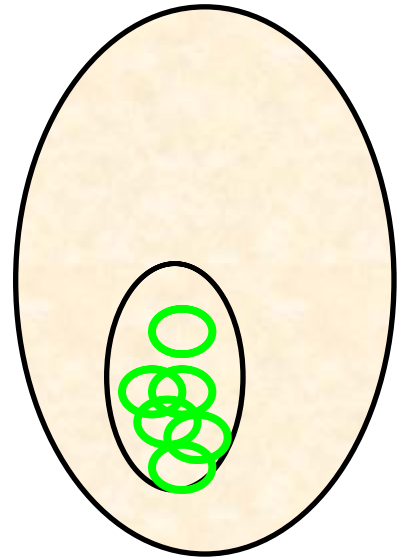
Distribution in population ?



Single copy (integrated)



multiple copy plasmids



GAL2	5.8	5.8
GAL3	0.17	0.17
GAL10	0.43	0.69
GAL80	0.42	0.41
Gfp	3.43	20.8
GAL4	0.22	0.11