Metabolic regulation
Using Evolution to understand genome structure and transcription regulation

Course Computational Biology 2025; Paulien Hogeweg; Theoretical Biology and Bioinformatics Grp Utrecht University

Present day (evolved) metabolism studied from evolutionary perspective

Last time:

By assuming optimality and equillibrium conditions and given the metabolic network of Yeast, genes kept in duplo after WGD could be "predicted"

TODAY

New insights in well studied metabolic regulation pathways by taking an evolutionaary perspective

Lac operon

Experimental and Modeling strategies

Experiments: use 'controlled conditions'

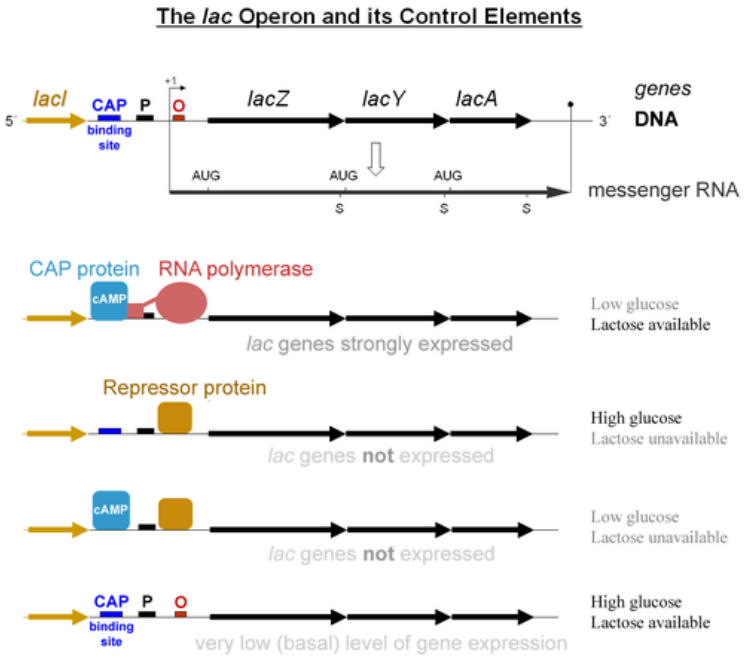
Mini-models: can study parameter space and 'choose' parameters based on outcome (fitting experiments)

Detailed models: use (MANY) measured / estimated ('reasonable') parameters

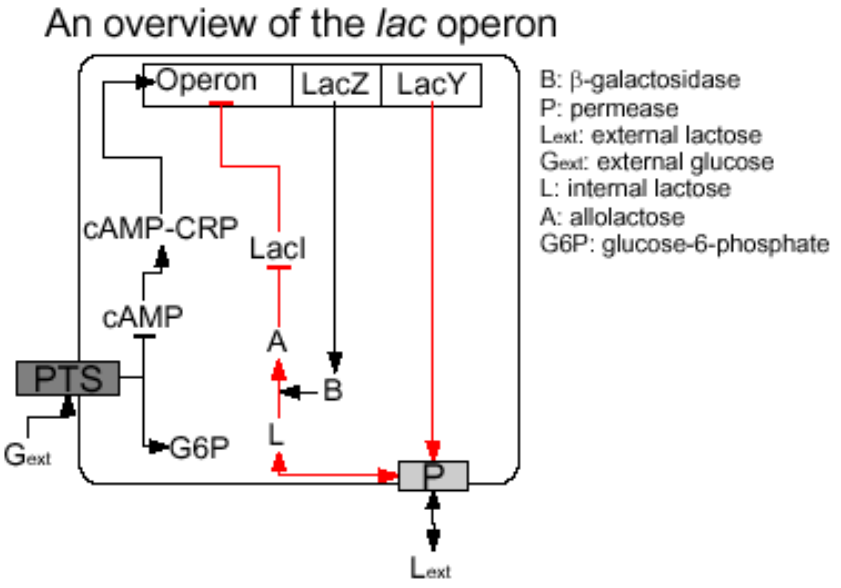
minimal evolutionary optimization models ('what is it good for?) (bet-hedging)

Here use multilevel (evolutionary) modeling to generate parameters and debug the above

Prototype gene regulation: Lac operon



genome structure



regulatory network

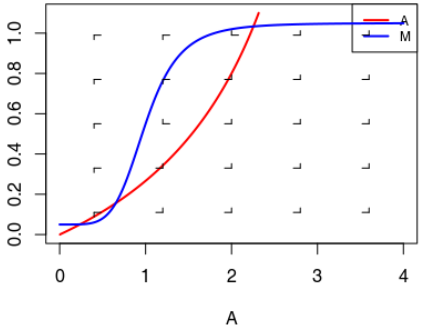
Lac Operon: Prototype bi-stability in gene regulation: classical mini-model, experiments

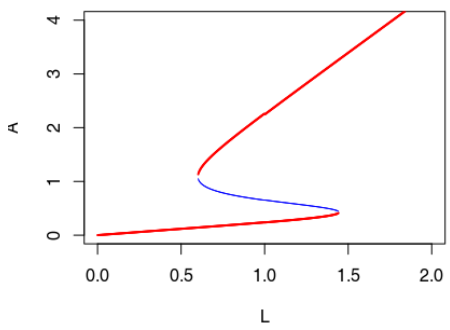
$$R = 1/(1 + A^n)$$

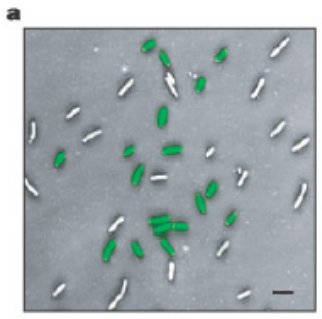
$$dM/dt = c_0 + c(1 - R) - dM$$

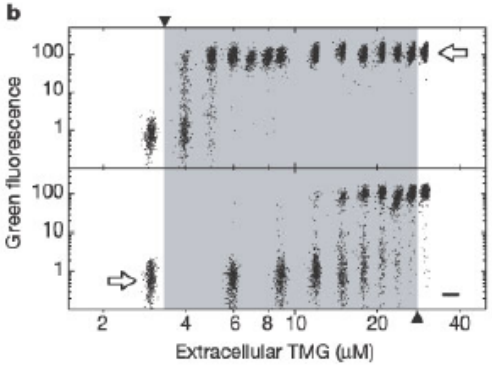
$$dA/dt = ML - \delta A - vMA$$

$$L = 1.0; c = 1.0; c_0 = .05; \delta = .2; v = .25; n = 5)$$







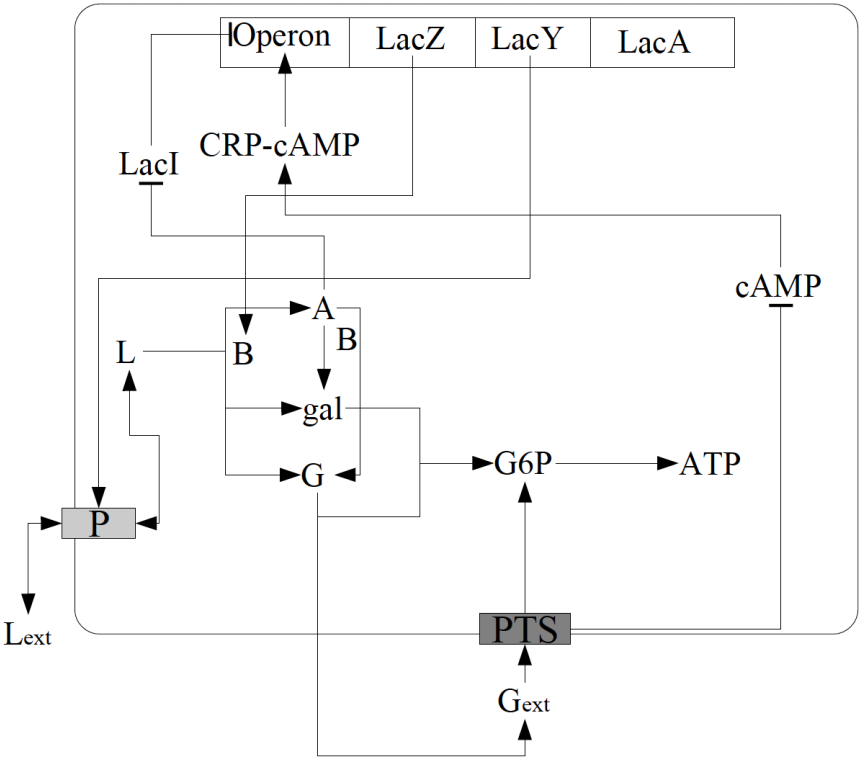


bi-stability

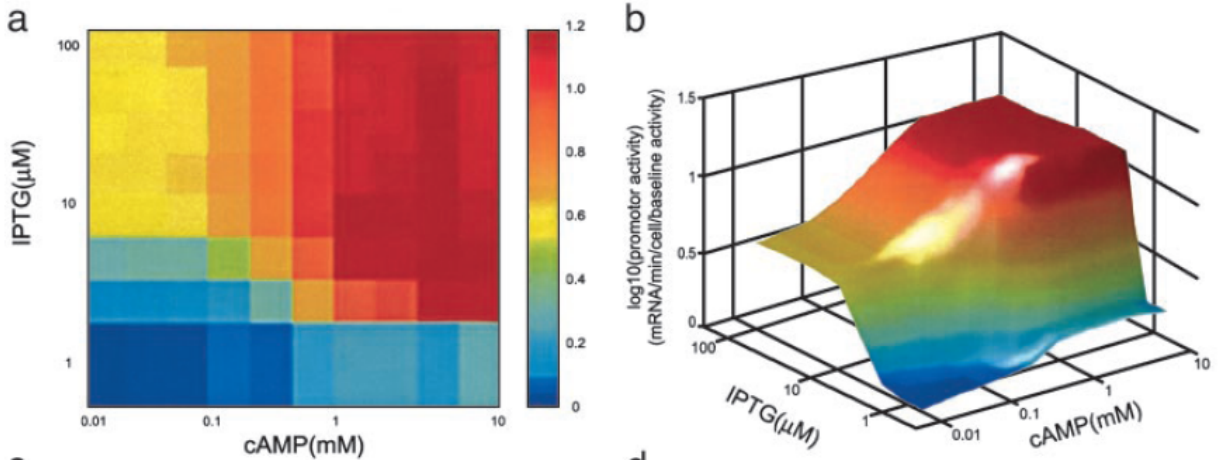
experimentally "verified"

cf Novick and Weiner 1957, Griffith 1968, Ozbudak et al 2004

Metabolic regulation in E.coli
Using Evolution to understand transcription regulation
Lac operon



experimental measurement of promotor function
Setty...Alon 2003



Not a simple AND function

"the wild-type region is selected to perform an elaborate computation in setting the transcription rate."

measurements fitted to model of promoter function

$$PA(A,C) = V_1 \frac{1 + V_2\mathcal{A} + V_3\mathcal{R}}{1 + V_4\mathcal{A} + V_5\mathcal{R}}, \tag{1}$$

where A stands for the allolactose concentration and C for the cAMP concentration and $\mathcal{A}$ and $\mathcal{R}$ are the fraction of active CRP and repressed LacI, respectively.

$$\mathcal{A} = \frac{(C/k_C)^n}{1 + (C/k_C)^n} \tag{2}$$

$$\mathcal{R} = \frac{1}{1 + (A/k_A)^m}, \tag{3}$$

where n and m are the Hill-coefficients of cAMP binding to CRP and allolactose binding to LacI. k_C and k_A are the dissociation constants for these reactions. Furthermore we have defined

$$\begin{aligned} V_1 &= (a\alpha + \gamma)/(1 + a) \\ V_2 &= d(b\beta + \gamma)/(a\alpha + \gamma) \\ V_3 &= \gamma c/(a\alpha + \gamma) \\ V_4 &= d(b + 1)/(a + 1) \\ V_5 &= c/(a + 1) \end{aligned} \tag{4}$$

$V_1 .... V_5$ depend on 7 affinity parameters

$a = RNAP/k_{RNAP}$, RNA-polymerase in units of its dissociation constant for binding to a free site.

$b = RNAP/k_{RNACP}$, RNA-polymerase in units of its dissociation constant for binding to a site with bound CRP.

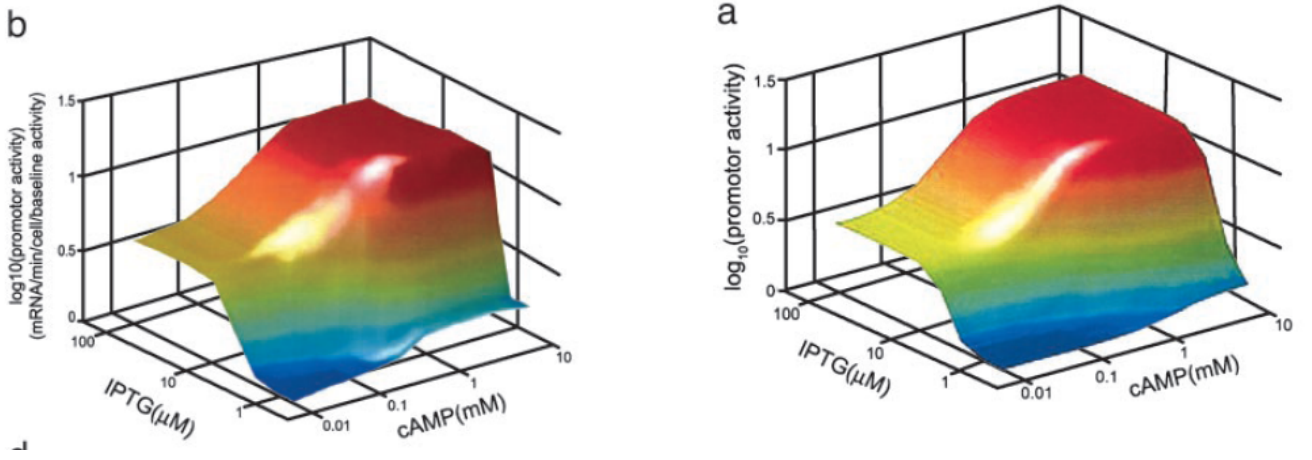
$c = LACI_T/k_{LACI}$, the total LacI concentration in units of its dissociation constant for binding to its site.

$d = CRP_T/k_{CRP}$, the total CRP concentration in units of its dissociation constant for binding to its site.

α, the transcription rate when RNA Polymerase is bound to the DNA, but CRP and LACI are not.

β, the transcription rate when both RNA Polymerase and CRP are bound, but LACI is not bound to the DNA.

γ, the "leakiness", the transcription rate when RNA Polymerase is not bound to the DNA.



data bestfit

Parameter sensitivity / parameter curse (1)

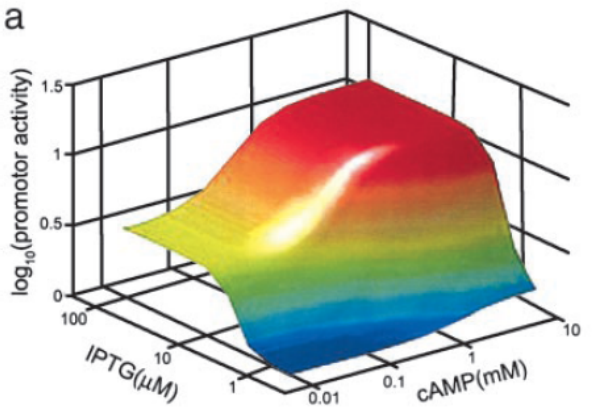
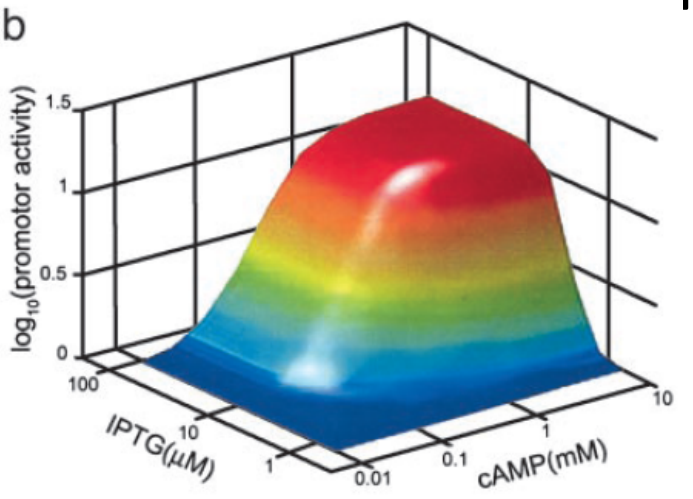
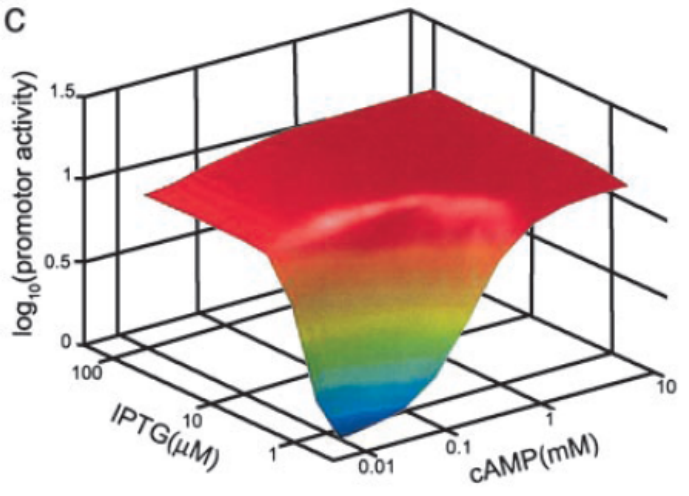


Table 1. ***lac*** **model parameters that best fit the measurement using the GFP reporter plasmid (wild type) and putative mutants that have purer AND-like and OR-like gates**

Parameter	Wild type	AND	OR
m	4 ± 0.6	4	4
n	2 ± 0.4	2	2
K_{IPTG}, μM	1.2 ± 0.2	1.2	1.2
K_{cAMP}, mM	1.8 ± 0.5	1.8	1.8
V_1	3.5 ± 0.7	**1**	**10**
V_2	70 ± 10	70	**1,700**
V_3	170 ± 30	**2,000**	**15**
V_4	17 ± 3	17	**400**
V_5	540 ± 100	**7,000**	**50**

AND





OR

more complex model of the lac operon

Wong et al 1997 , adapted by van Hoek & Hogeweg 2006

$$
\begin{aligned}
PA(A,C) &\equiv V_1 \frac{1 + \frac{V_2 (C/k_C)^n}{1+(C/k_C)^n} + \frac{V_3}{1+(A/k_A)^m}}{1 + \frac{V_4 (C/k_C)^n}{1+(C/k_C)^n} + \frac{V_5}{1+(A/k_A)^m}} \\
\frac{dM}{dt} &= P(A,C) - (\gamma_M + \mu) M \\
\frac{dB}{dt} &= k_B M - (\gamma_B + \mu) B \\
\frac{dP}{dt} &= k_P M - (\gamma_P + \mu) P
\end{aligned}
$$

eqs determining operon activity

$$
\begin{aligned}
PA(A,C) &= V_1 \frac{1 + V_2 \frac{(C/k_C)^n}{1+(C/k_C)^n} + \frac{V_3}{1+(A/k_A)^m}}{1 + V_4 \frac{(C/k_C)^n}{1+(C/k_C)^n} + \frac{V_5}{1+(A/k_A)^m}} \\
\frac{dM}{dt} &= PA(A,C) - (\gamma_M + \mu)M \\
\frac{dB}{dt} &= k_B M - (\gamma_B + \mu)B \\
\frac{dP}{dt} &= k_P M - (\gamma_P + \mu)P \\
\frac{dL}{dt} &= P\frac{k_{L,i} L_{ext}}{K_{L,i} + L_{ext}} - P\frac{k_{L,o} L}{K_{L,o} + L} \\
&\quad -B\frac{(k_{c,L} + k_{L-A})L}{L + K_{m,L}} - (\gamma_L + \mu)L \\
\frac{dA}{dt} &= B\frac{k_{L-A} L}{L + K_{m,L}} - B\frac{k_{c,A} A}{A + K_{m,A}} - (\gamma_A + \mu)A
\end{aligned}
$$

eqs determining further metabolism and cell growth(X)

(cell division if cell size = 2*basic size)

$$
\begin{aligned}
\frac{dG}{dt} &= \frac{k_{c,L}B*L}{L+K_{m,L}} + \frac{k_{c,A}B*A}{A+K_{m,A}} - \frac{k_{c,G}G}{G+K_{m,G}} - k_{G,o}(G-G_{ext}) - \mu G \\
\frac{dG6P}{dt} &= \frac{k_{t,G}G_{ext}}{G_{ext}+K_{t,G}} + \frac{k_{c,G}G}{G+K_{m,G}} + \frac{k_{c,L}B*L}{L+K_{m,L}} + \frac{k_{c,A}B*A}{A+K_{m,A}} - \\
&\quad \frac{k_{G6P,R}G6P}{G6P+K_{G6P,R}} - \frac{k_{G6P,F}G6P^8}{K_{G6P,F}^8+G6P^8} - \mu G6P \\
\frac{dC}{dt} &= \frac{k_{s,C}K_{s,C}}{\frac{k_{t,G}G_{ext}}{G_{ext}+K_{t,G}}+K_{s,C}} - (\gamma_C+\mu)C \\
\frac{dATP}{dt} &= \frac{Y_R*k_{G6P,R}*G6P}{G6P+K_{G6P,R}} + \frac{2k_{G6P,F}*G6P^8}{K_{G6P,F}^8+G6P^8} - BMC - \\
&\quad \frac{\mu_{max}*GC*ATP^4}{ATP^4+K_{ATP}^4} - PC*PA - \frac{k_{c,L}B*L}{L+K_{m,L}} - \frac{k_{c,A}B*A}{A+K_{m,A}} \\
\frac{dX}{dt} &= \mu_{max}\frac{ATP^4}{ATP^4+K_{ATP}^4}X
\end{aligned}
$$

Wong concluded : bistable switch

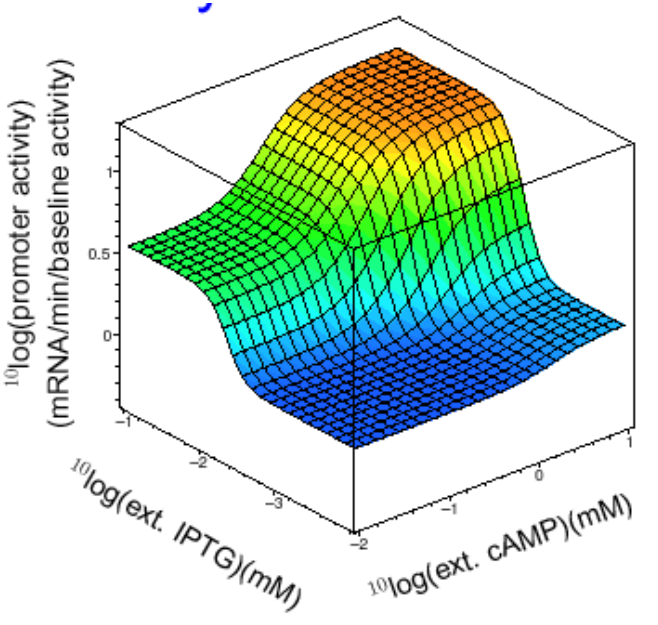
Table 1: All model parameters with their values.

parameter	equation	value	comments
k_C	Eq. 2	evolvable, mM	initial value: 1.0×10^{-3} mM
n	Eq. 2	evolvable	initial value: 4.0
k_A	Eq. 3	evolvable, mM	initial value: 5.5×10^{-4} mM
m	Eq. 3	evolvable	initial value: 8.0
a	Eq. 4	evolvable	initial value: 1.0
b	Eq. 4	evolvable	initial value: 1.0
c	Eq. 4	evolvable	initial value: 1.0×10^{6}
d	Eq. 4	evolvable	initial value: 50
α	Eq. 4	evolvable, mM/min	initial value: 1.1×10^{-7} mM/min
β	Eq. 4	evolvable, mM/min	initial value: 2.2×10^{-5} mM/min
γ	Eq. 4	evolvable, mM/min	initial value 1.1×10^{-9} mM/min
γ_M	Eq. 5	0.693/min	Wong et al. (2)
k_B	Eq. 6	9.4 mM enzyme/(mM mRNA min)	Wong et al. (2)
γ_B	Eq. 6	0.01/min	Wong et al. (2)
k_P	Eq. 7	18.8 mM enzyme/(mM mRNA min)	Wong et al. (2)
γ_P	Eq. 7	0.01/min	Wong et al. (2)
$k_{Lac,in}$	Eq. 8	2148 mmol lactose/(mmol permease min)	Wong et al. (2)
$K_{Lac,in}$	Eq. 8	0.26 mM	Wong et al. (2)
$k_{Lac,out}$	Eq. 8	2148 mmol lactose/(mmol permease min)	Wong et al. (2)
$K_{Lac,out}$	Eq. 8	0.26 mM	unlike Wong et al. (2), intracellular concentrations are in mM
$k_{Lac-Allo}$	Eq. 9	8460/min	Wong et al. (2)
$K_{m,Lac}$	Eq. 9, Eq. 10	1.4 mM	Martinez-Bilbao et al. (13), referred to by Wong et al. (2)
$k_{cat,Lac}$	Eq. 10	9540/min	Wong et al. (2)
γ_L	Eq. 11	0.15/min	assumed, to get a significant bistable region, compare Yildirim and Mackey (4)
$k_{cat,Allo}$	Eq. 12	18000/min	Wong et al. (2)
$K_{m,Allo}$	Eq. 12	0.28 mM	Wong et al. (2)
γ_A	Eq. 13	0.15/min	assumed, to get a significant bistable region, compare Yildirim and Mackey (4)
$k_{cat,Glu}$	Eq. 14	11.5 mM/min	fitted with data of Hogema et al. (6)
$K_{m,Glu}$	Eq. 14	0.45 mM	fitted with data of Hogema et al. (6)
$k_{Glu,out}$	Eq. 15	0.693/min	fitted with data of Hogema et al. (6)
$k_{t,Glu}$	Eq. 15	45 mM/min	Wong et al. (2), Carlson and Srienc (11)
$K_{t,Glu}$	Eq. 15	0.015 mM	Wong et al. (2)

parameter	equation	value	comments
$k_{G6P,Rsp}$	Eq. 18	34 mM/min	assumed, saturated respiratory flux assumed for maximal glucose influx. Andersen and Von Meyenburg (10)
$K_{G6P,Rsp}$	Eq. 18	0.5 mM	idem. Andersen and Von Meyenburg (10)
$k_{G6P,Frm}$	Eq. 19	200 mM/min	assumed, maximal fermentative flux is much larger than maximal respiratory flux. Andersen and Von Meyenburg (10)
$K_{G6P,Frm}$	Eq. 19	20 mM	assumed, fermentation saturates much slower than respiration. Andersen and Von Meyenburg (10)
$k_{syn,cAMP}$	Eq. 21	0.001 mM/min	Wong et al. (2)
$K_{syn,cAMP}$	Eq. 21	1.0 mM/min	assumed, to have a large range of possible cAMP concentrations.
γ_{cAMP}	Eq. 21	2.1/min	Wong et al. (2)
Y_{RsP}	Eq. 22	32 mM ATP/mM glucose-6-phosphate	assumed equal to the ATP-yield of aerobic respiration.
BMC	Eq. 22	23.5 mM/min	Carlson and Srienc (11)
GC	Eq. 22	7.28×10^5 mM	estimated with data of Carlson and Srienc (11)
PC	Eq. 22	2.36×10^6 mM ATP/mM mRNA	calculated assuming 3% growth cost at maximal activity, Koch (14). (for high cost a value ten times higher is used)
μ_{max}	Eq. 23	0.0233/min	Wong et al. (2)
Q	Eq. 25, Eq. 26	0.00035	assumed
D		$0.0020(gridsize)^2/min$	assumed, scalable
Δ_a		0.075	assumed
Δ_b		0.075	assumed
Δ_c		0.15	assumed
Δ_d		0.15	assumed
Δ_α		0.075	assumed
Δ_β		0.075	assumed
Δ_γ		0.075	assumed
Δ_{k_A}		0.15	assumed
Δ_{k_C}		0.05	assumed
Δ_n		0.5	assumed
Δ_m		0.5	assumed
$V_{mRNA,max}$		2.2×10^{-5} mM/min	assumed, to have a realistic maximal lactose uptake rate.

Functionality of Lac-operon
Bistability?

- Most studied regulatory system
- often considered as AND gate
  ON if lactose and not glucose;
  otherwise OFF
- recent direct promoter measurements:
  more graded response
- Bistability? exp 'seen' and expected
  from minimodels and 'verified' in more
  extensive parametrized models
- many (all) parameters measured
  HOWEVER
  may be orders of magnitude different
  parameter curse (2)!



Setty .... Alon 2003

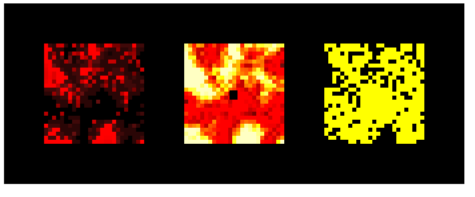
Does such a promotor function evolve
DOES BISTABILITY EVOLVE?; alleviate parameter uncertainty

van Hoek and Hogeweg, BJ 2006 PLOS Comp biol 2007

"experimental setup" evolution of the Lac operon: timescales: metabolism, cell growth/division, prot. stab, environmental switches, evolution

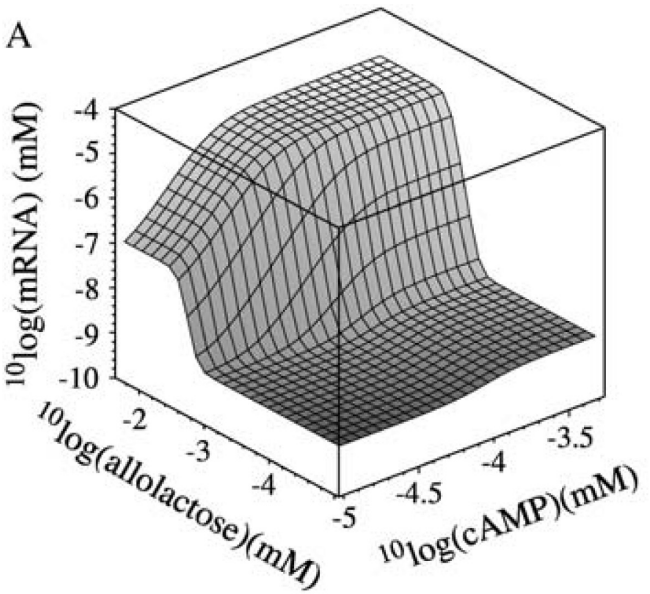
- Adapt existing detailed quantitative model of lac operon dynamics (Wong et al 1997)
- use measured parameters EXCEPT for lac operon parameters
- evolve 11 lac-operon parameters
  DO NOT use dimension reduction!
  otherwise evolutionary lock-in

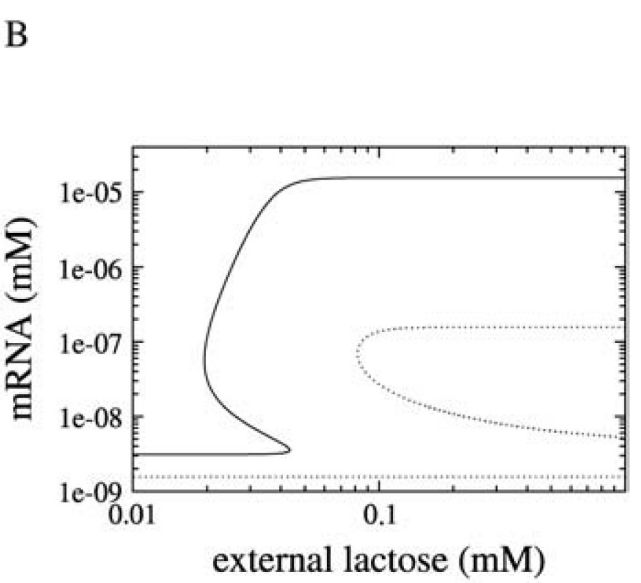
- Design environment! ("cover all possibilities")
- global/aperiodic influx of lactose and glucose in medium, diffusion, scaling
- growth (dependent on ATP), division (2*size), decay (density dep; no ATP)
- encountered environments depend on dynamics! dynamics!



evolution as trick to cope with parameter uncertainty

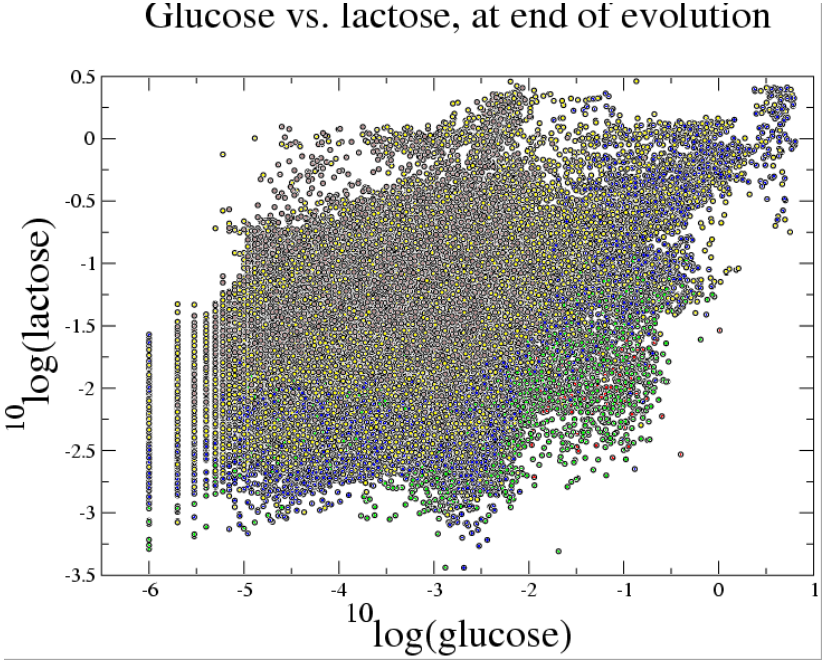
initialize as a bistable switch
(because no bistable switch evolved...)

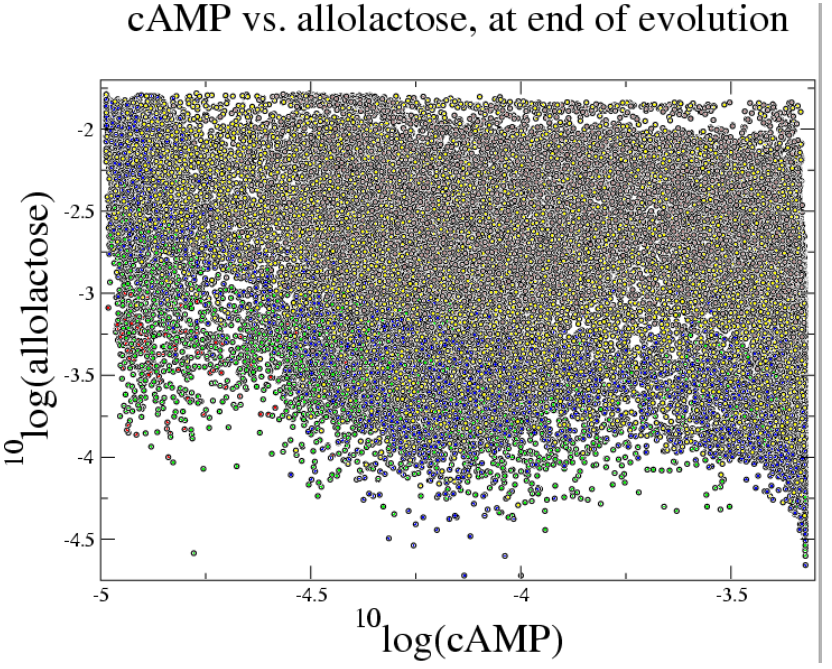




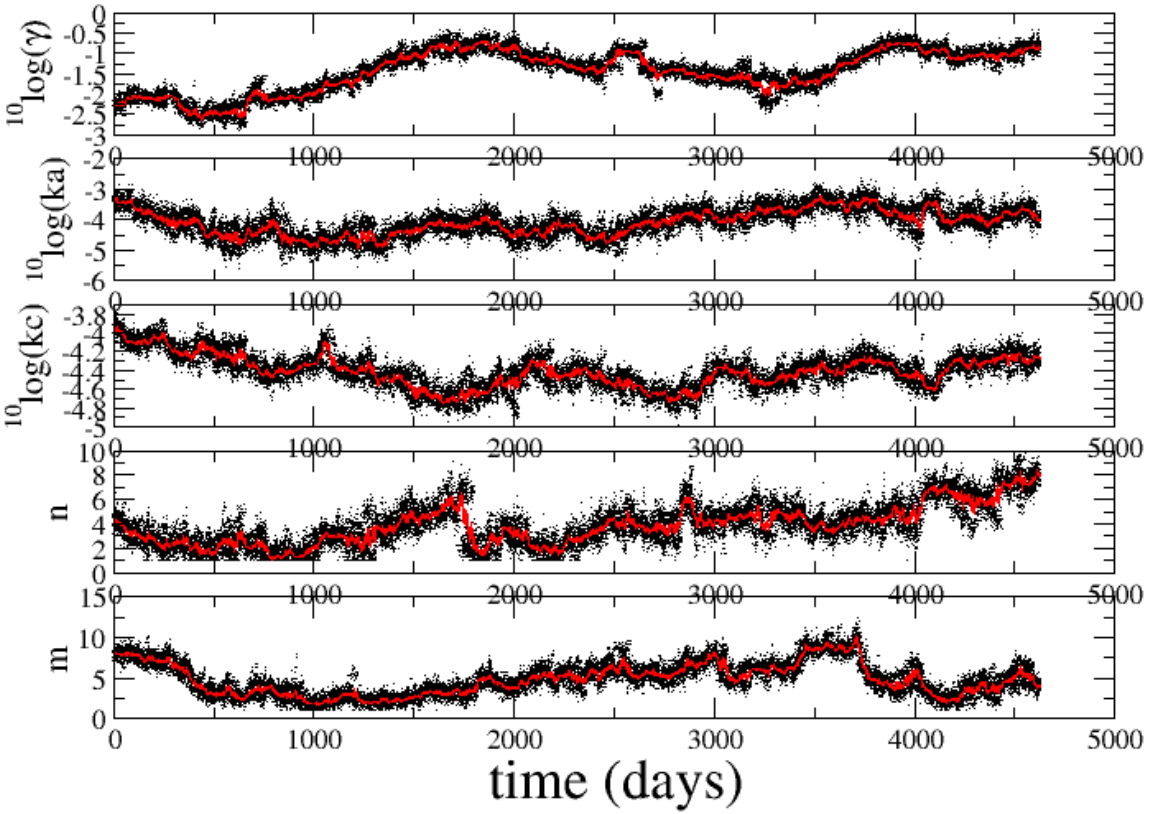
solid low glucose; dotted high glucose

Designing external environment coverage of environmental statespace, while response to environments

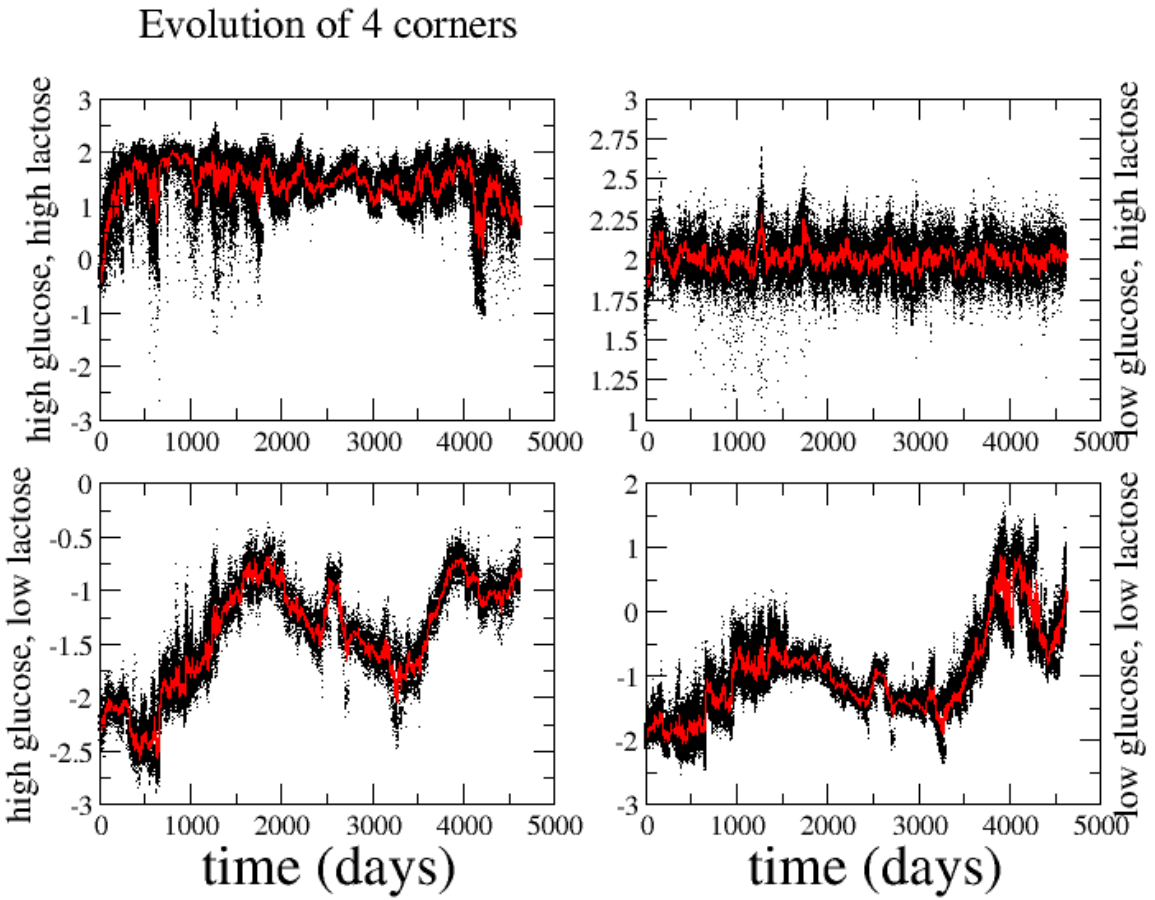




Evolution: how to observe parameter of individuals in pop. in time



Evolution: how to observe phenotypic features in time (4 extremes)



Evolution: how to observe comparison of evolutionary outcomes

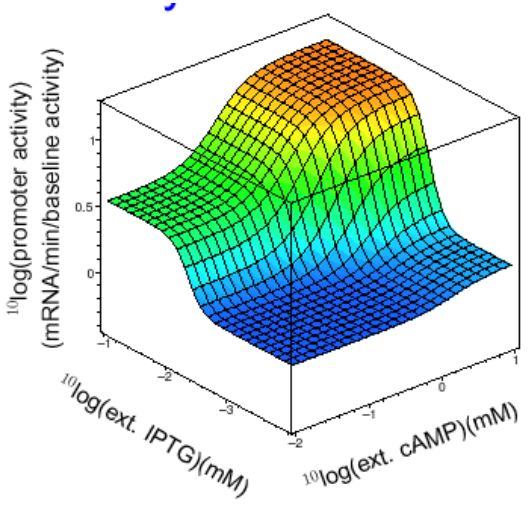
Ancestor trace!

Compete last common ancestors (n*)

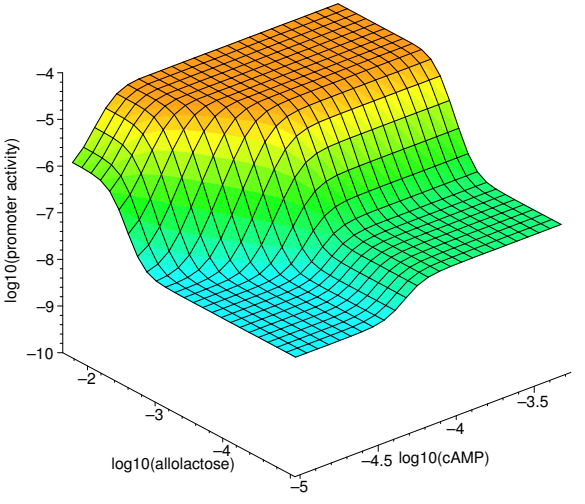
Compete last populations (n*)

$-->$ "BEST" evolved promoter function

'Best' evolved last common ancestor deterministic intracellular dynamics; 11hr average influx regime spatial pattern formation

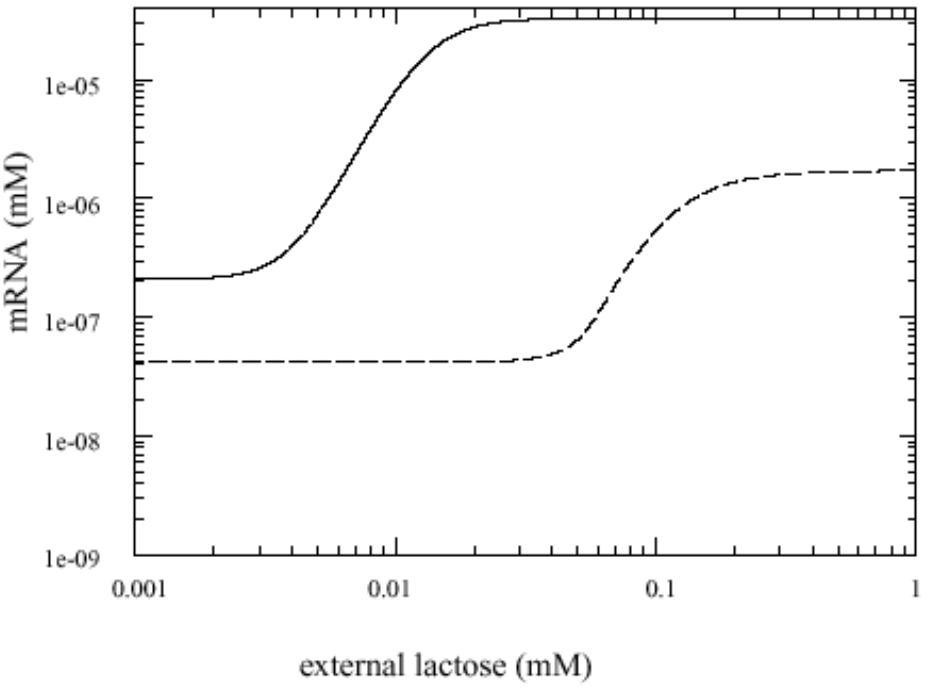


OBSERVED (Setty 2003)



BEST EVOLVED LCA

Similar to measured promoter function
However NO bistability



What about experiments / prior modeling?

Conditions for bistability for artificial inducer VERY different from those for lactose.

$$\lambda(C) \equiv \frac{PA(0,C)}{(m-1)^2}\left(\frac{(m+1)^2}{PA(\infty,C)} + 4m\zeta\right) < 1.$$

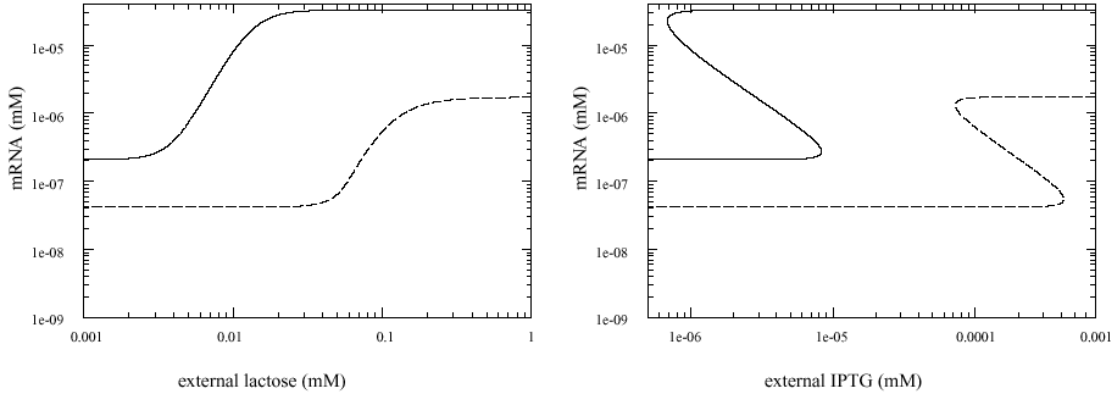
for lactose:

$$\frac{PA(0,C)4m\zeta}{(m-1)^2} < 1.$$

for artificial inducer

$$\frac{PA(0,C)}{(m-1)^2}\frac{(m+1)^2}{PA(\infty,C)} < 1.$$

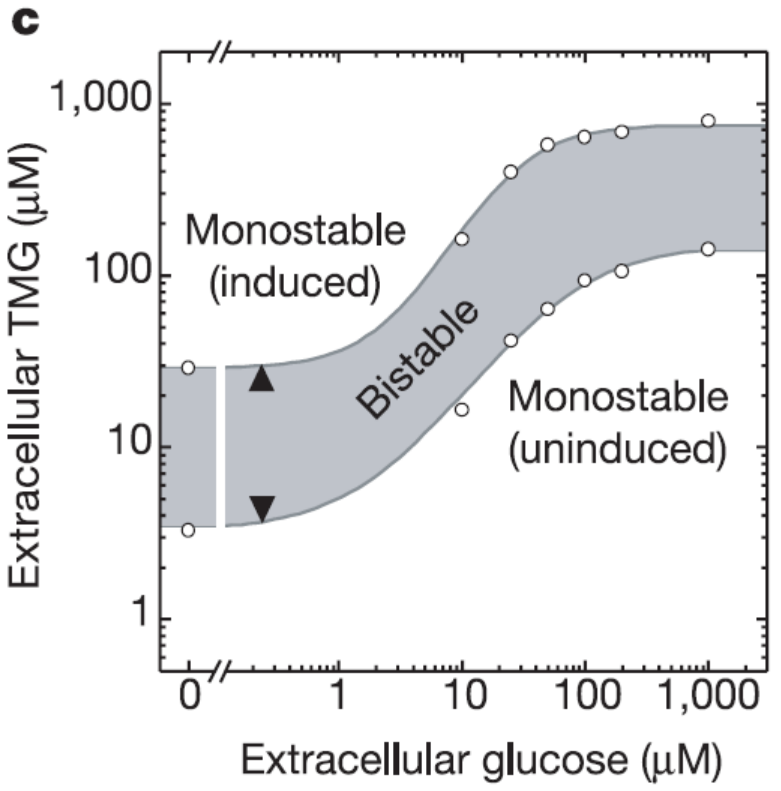
Evolved promoter function bistable for artificial inducer!

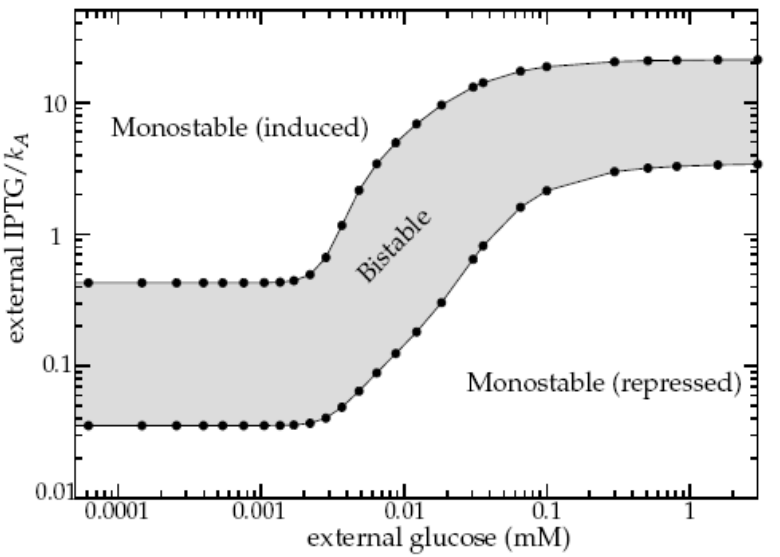


LACTOSE

ART INDUCER

evolved vs measured bistability for artificial inducer

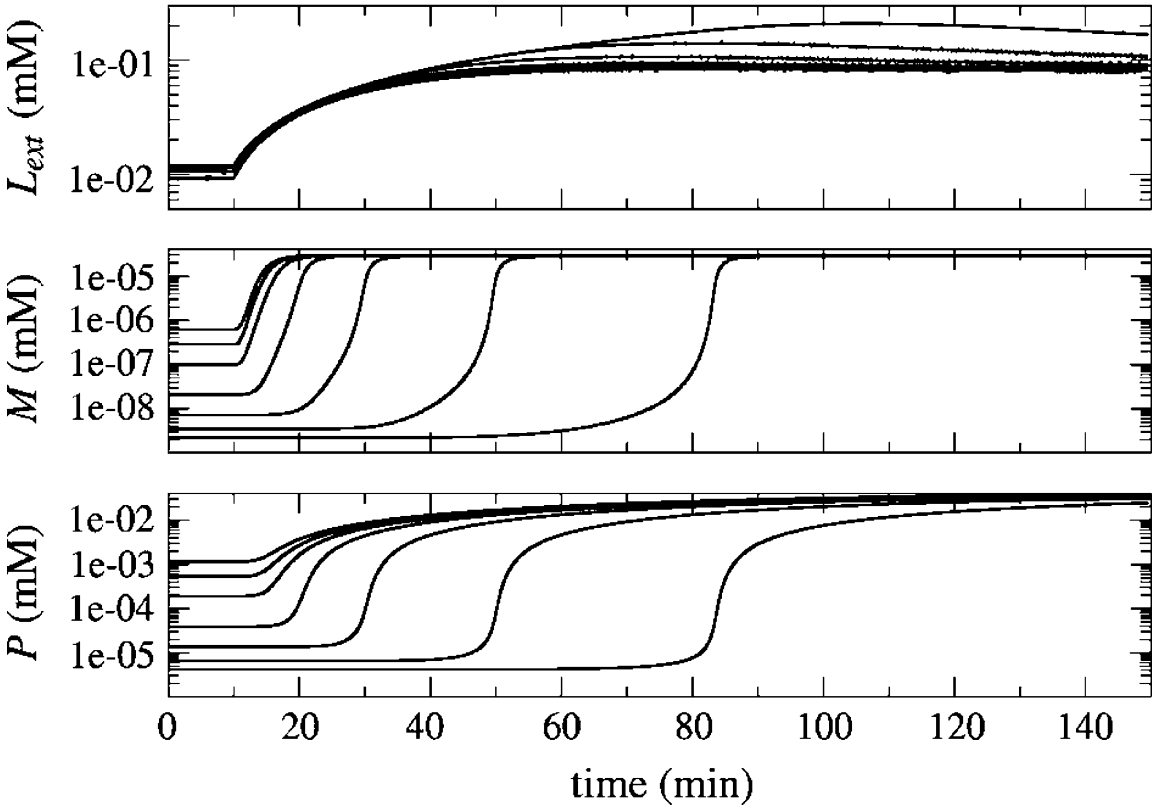




measured (van Oudenaarden)

evolve (van Hoek)

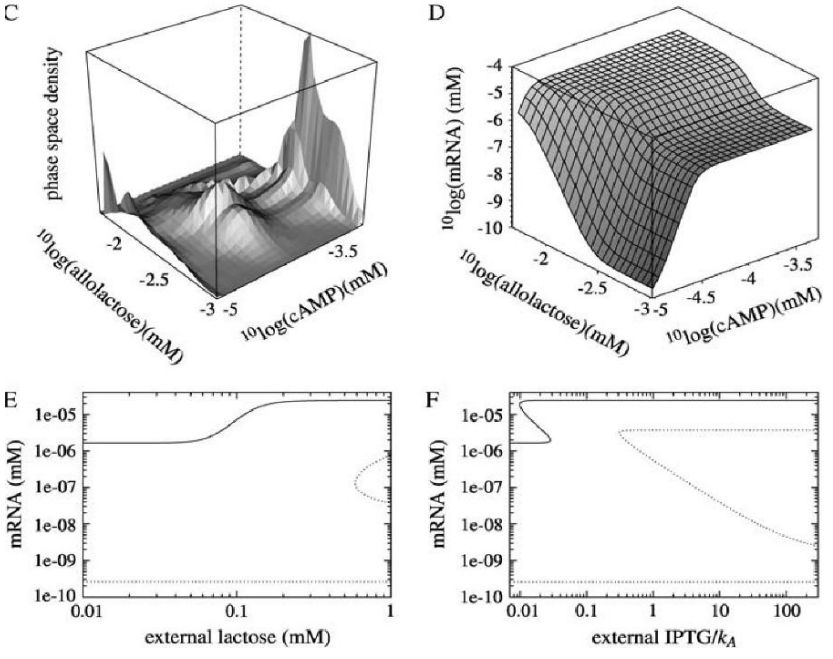
Why avoid bistability
why 'waste' expression when no (low) Lactose available
Non-equilibrium: delays!

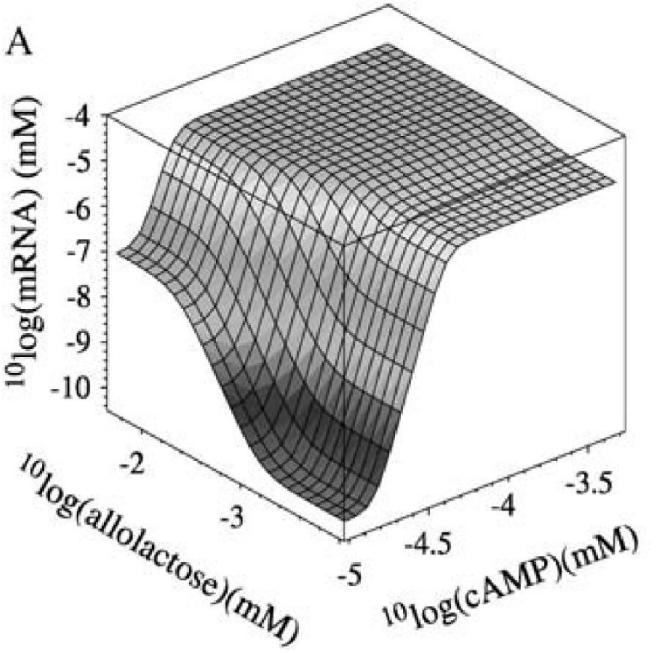


lines for different γ values (P(0,C))

(E.coli division time ca 1hr)

sensitivity to experimental design
cost of expression and frequency of environmental switching





high cost bistab at rare high glucose

fast switch : loss of regulation

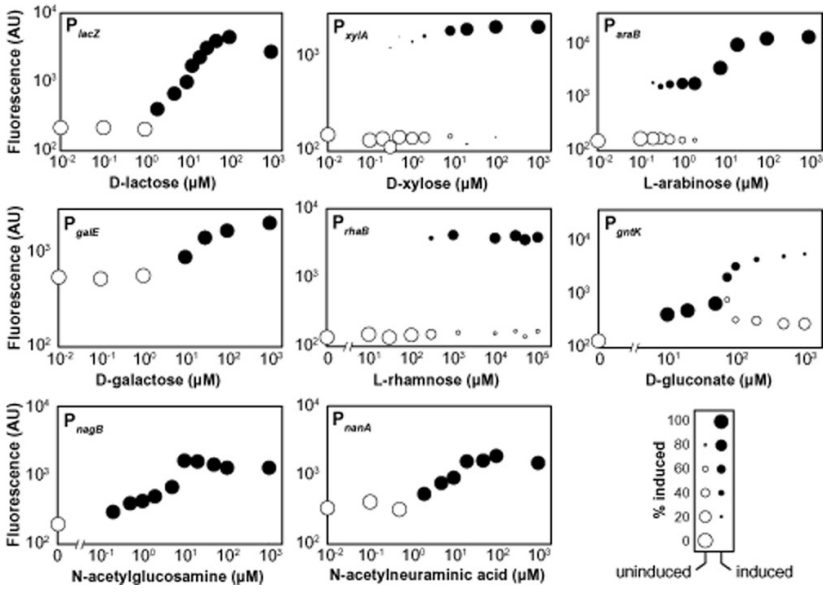
Experimental support for evolutionary model

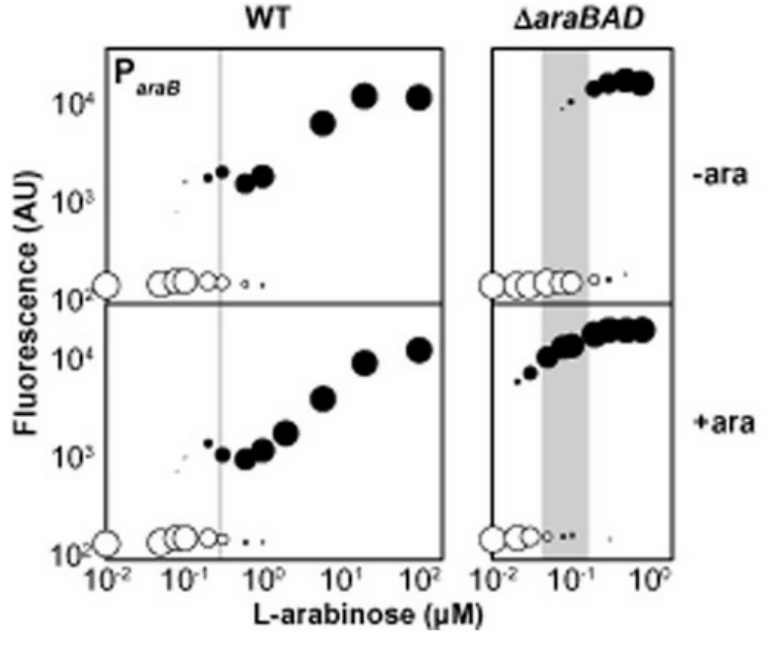
E.M. Ozbudak, M. Thattai, H.N. Lim, B.I. Shraiman, A. Van Oudenaarden Multistability in the lactose utilization network of Escherichia coli. Nature, 427 (2004), pp. 737

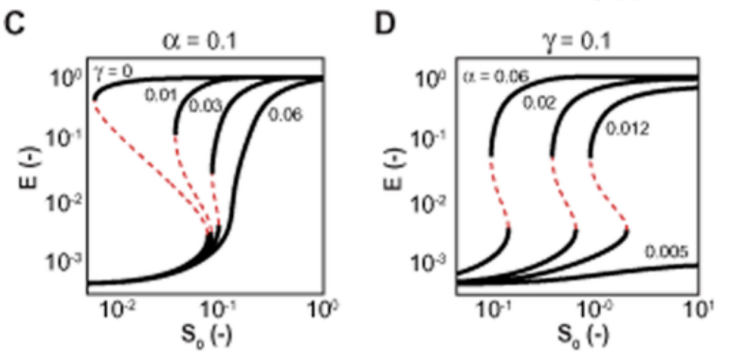
in supplementary material

During induction with lactose, *as opposed to IPTG, TMG*.....
the steady state distribution after 4 hours of growth is always uni-modal, and we never observe hysteresis.

various responses for different sugars
suppressing catabolism enhances hysteresis



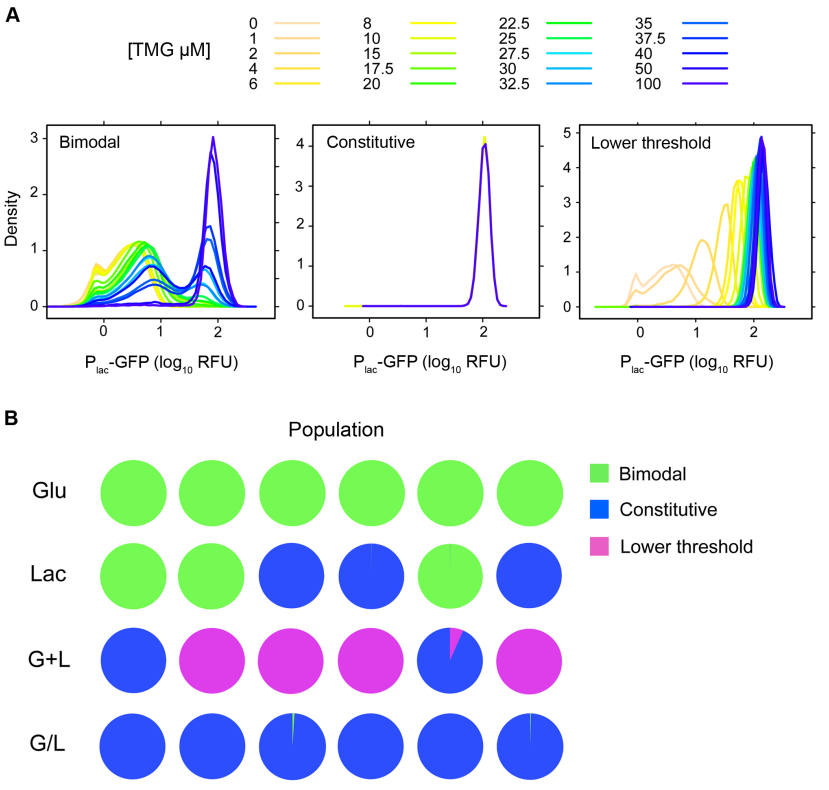




Afroz et al 2014

experimental support of fast evolutionary change, avoidance of bistability even relative to TMG

cf Adaptive Evolution of the Lactose Utilization Network in Experimentally Evolved Populations of Escherichia coli Quan et al 2012



Biophysical Journal
Article

Biophysical Society

Bistability and Nonmonotonic Induction of the *lac* Operon in the Natural Lactose Uptake System

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ABSTRACT The *Escherichia coli lac* operon is regulated by a positive feedback loop whose potential to generate an all-or-none response in single cells has been a paradigm for bistable gene expression. However, so far bistable *lac* induction has only been observed using gratuitous inducers, raising the question about the biological relevance of bistable *lac* induction in the natural setting with lactose as the inducer. In fact, the existing experimental evidence points to a graded rather than an all-or-none response in the natural lactose uptake system. In contrast, predictions based on computational models of the lactose uptake pathway remain controversial. Although some argue in favor of bistability, others argue against it. Here, we reinvestigate *lac* operon expression in single cells using a combined experimental/modeling approach. To this end, we parameterize a well-supported mathematical model using transient measurements of LacZ activity upon induction with different amounts of lactose. The resulting model predicts a monostable induction curve for the wild-type system, but indicates that overexpression of the LacI repressor would drive the system into the bistable regime. Both predictions were confirmed experimentally supporting the view that the wild-type *lac* induction circuit generates a graded response rather than bistability. More interestingly, we find that the *lac* induction curve exhibits a pronounced maximum at intermediate lactose concentrations. Supported by our data, a model-based analysis suggests that the nonmonotonic response results from saturation of the LacI repressor at low inducer concentrations and dilution of Lac enzymes due to an increased growth rate beyond the saturation point. We speculate that the observed maximum in the lac expression level helps to save cellular resources by limiting Lac enzyme expression at high inducer concentrations.

model and parameters used (literature + measured) ((slightly) different to previous model)

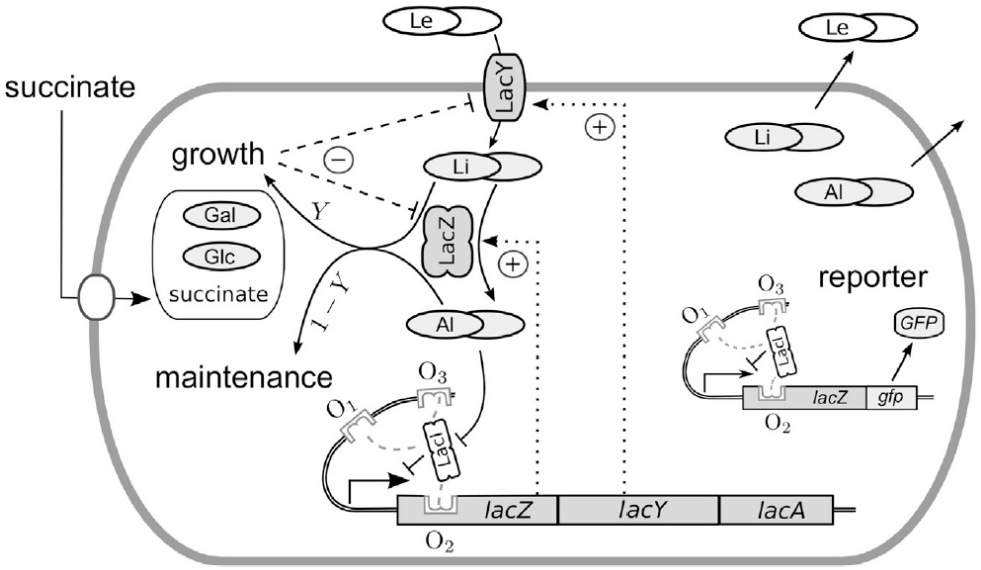
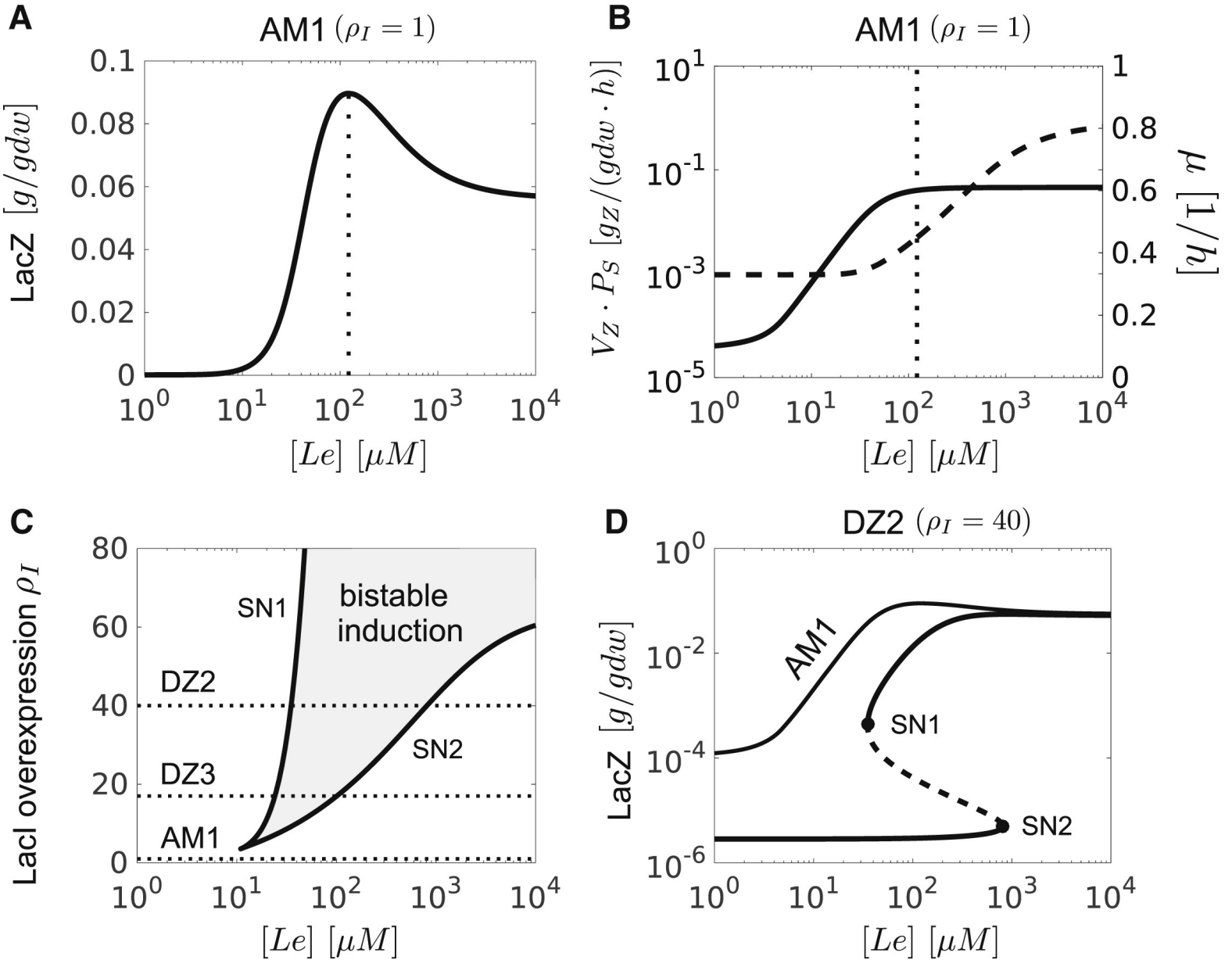


TABLE 2 Model Parameters

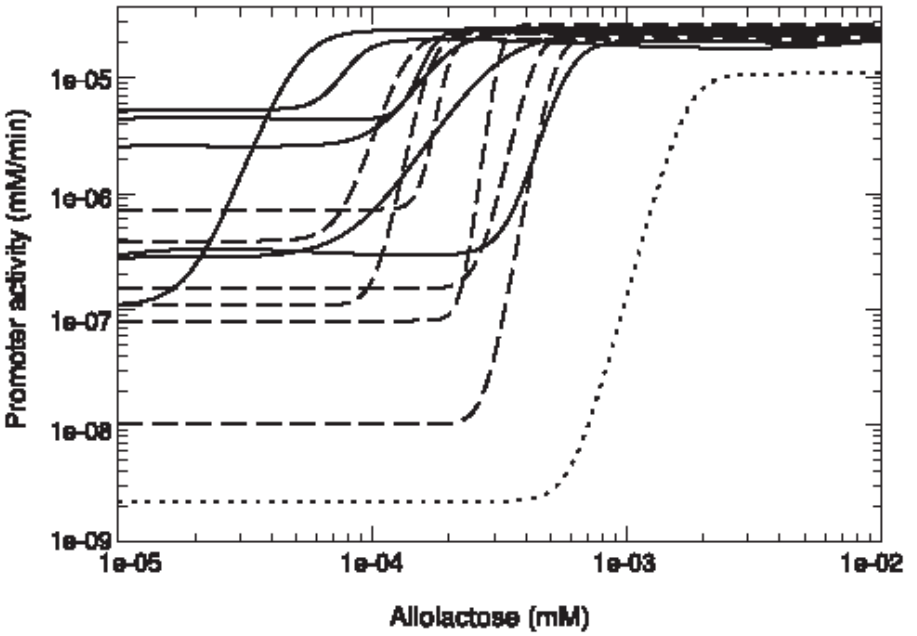
Name	Value	Reference	Name	Value	Reference
V_L	$1271\frac{g_L/\text{gdw}}{g_Y/\text{gdw}}\frac{1}{\text{h}}$	(20)	κ_2	0.38	(34)
K_L	0.68 g_L/L ($\approx$2 mM)	estimated	α_1	31	(34)
K_i	$0.00013\frac{g_L}{\text{gdw}}$	estimated	$\widehat{\alpha}_1$	1420	(34)
V_{lac}	$670\frac{g_L/\text{gdw}}{g_Z/\text{gdw}}\frac{1}{\text{h}}$	(30)	α_2	19	(34)
K_{lac}	$0.0029\frac{g_L}{\text{gdw}}$	(30)	$\widehat{\alpha}_2$	322	(34)
V_{al}	$1019\frac{g_A/\text{gdw}}{g_Z/\text{gdw}}\frac{1}{\text{h}}$	(30)	α_3	3	(34)
K_{al}	$0.0014\frac{g_A}{\text{gdw}}$	(30)	Y	$0.092\frac{g_L}{\text{gdw}}$	estimated
V_Z	$0.046\frac{g_Z/\text{gdw}}{\text{h}}$	(28)	k_e^-	$60\frac{1}{\text{h}}$	(21)
K_a	$1.5 \times 10^6\frac{1}{g_A/\text{gdw}}$	(27)	μ_b	$0.33\frac{1}{\text{h}}$	measured

g_X/gdw denotes gram of species $X = L, A, Y, Z$ per gram dry weight (see Table 1).

No bistability for measured parameters, but can be induced by over-expression of LacI. LacZ expression saturates, and by dilution LacZ concentration peaks at intermediate Le, because growth rate increases. Zander et al 2017


A
AM1 ($\rho_I = 1$)
LacZ $[g/gdw]$
0.1
0.08
0.06
0.04
0.02
0
10^0 10^1 10^2 10^3 10^4
$[Le]$ $[\mu M]$
B
AM1 ($\rho_I = 1$)
$V_Z \cdot P_S$ $[gz/(gdw \cdot h)]$
10^1
10^{-1}
10^{-3}
10^{-5}
μ $[1/h]$
1
0.8
0.6
0.4
0.2
0
10^0 10^1 10^2 10^3 10^4
$[Le]$ $[\mu M]$
C
LacI overexpression ρ_I
80
60
40
20
0
SN1
bistable induction
DZ2
SN2
DZ3
AM1
10^0 10^1 10^2 10^3 10^4
$[Le]$ $[\mu M]$
D
DZ2 ($\rho_I = 40$)
LacZ $[g/gdw]$
10^0
10^{-2}
10^{-4}
10^{-6}
AM1
SN1
SN2
10^0 10^1 10^2 10^3 10^4
$[Le]$ $[\mu M]$

Indeed in the model evolution of lac operon avoids bistability by increasing repressed expression level (and even more so in stochastic version)



dotted: start (bistable); solid evolved stoch.; dashed evolved det.

evolved in well mixed system without glucose

conclusions
Evolutionary modeling to 'test' regular systems biology models/experiments

---

Evolutionary perspective helped to debug long held misconceptions

which were prior "verified" theoretically AND experimentally

Evolutionary modeling powerful tool for alleviating parameter uncertainty

Evolutionary change in parameters very uninformative

Parameter uncertainty inherent in evolutionary context
(parameter redundancy; condition dependent parameter change
("TRUE" parameters do not exist))

Non-supervised modeling 'fits' better then fitted supervised models

Modeling gene regulation/signal transduction
Monster of Loch Ness syndrome

"quod erat demonstrandum"

evolution as trick to cope with parameter uncertainty

HOWEVER: "function" of bistability is often assumed increased population variability, and therewith rapid adaptation, GIVEN stochastic gene expression

Above results artifact of deterministic modeling?

study: bistability and stochasticity
in the lac operon

cf Thattai & van Oudenaarden (2004):
noise + bistability can be 'good' because it allows rapid switching due to population heterogeniety.

However: minimization of expression noise in essential genes (Fraser et al 2004)

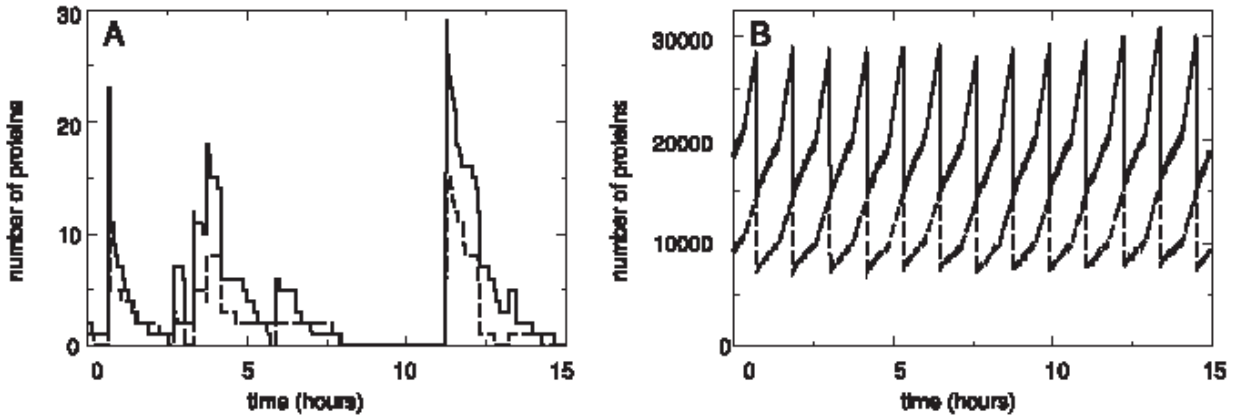
But: Excessive stochasticity of promoter function measured in E. coli (Wolf & van Nimwegen 2016)

from deterministic to stochastic model of lac operon only one (measured) parameter added

protein translation occurs in bursts:
geometrically distribution, average size 5 proteins
(Cai et al 2007)

model chance of burst proportional to # mRNA

at cell division distribute proteins binomially over the cells

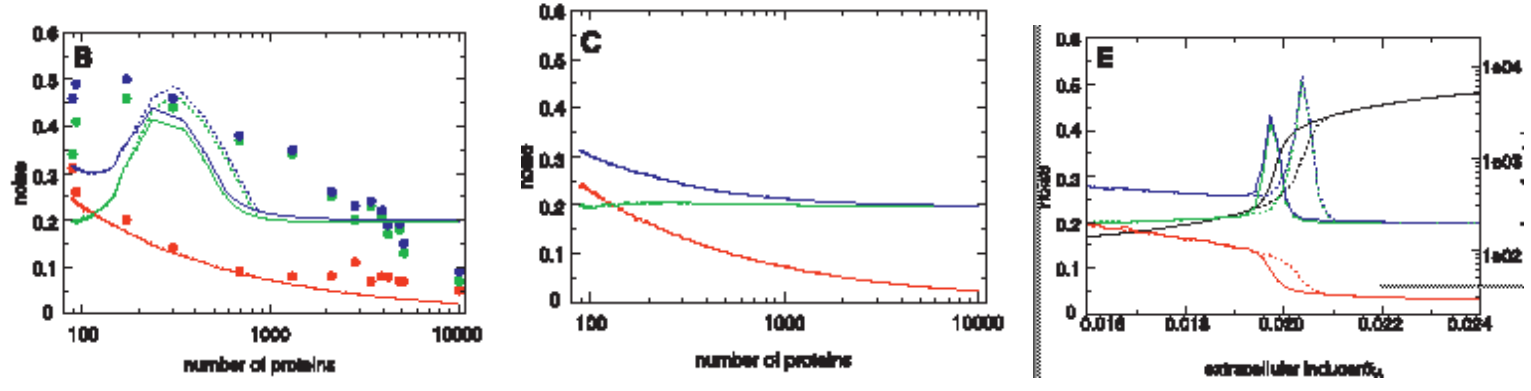


intrinsic vs extrinsic noise: experiments and model

extrinsic noise: cell cycle+ intracellular inducer concentration (green)

intrinsic noise: difference in expression of 2 identical promotors in a single cel (red)

$N_{tot} = N_{ext} + N_{int} = std/mean$ in population (blue)

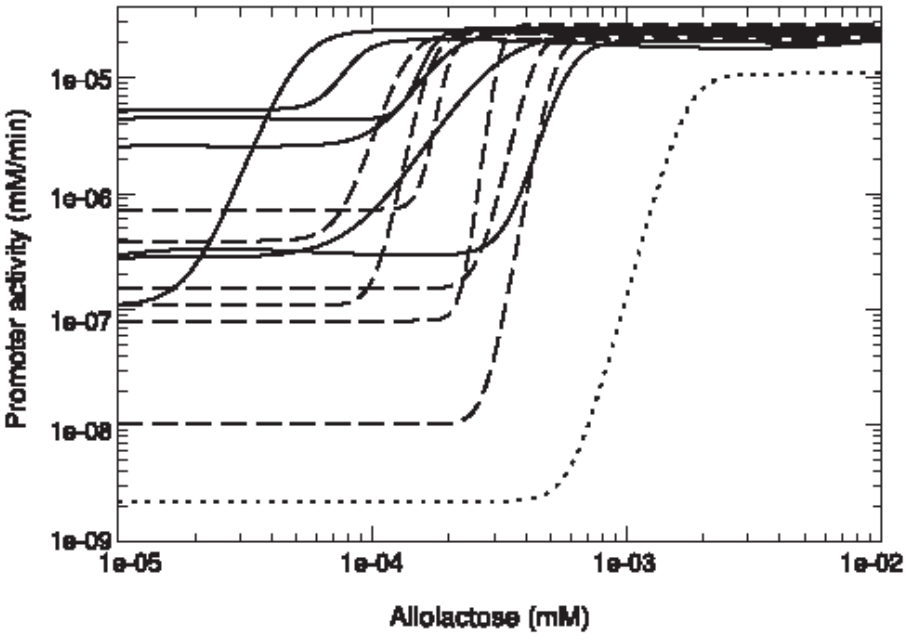


IPTG as inducer Lac as inducer IPTG as inducer

noise relative to internal protein numbers relative to external IPTG

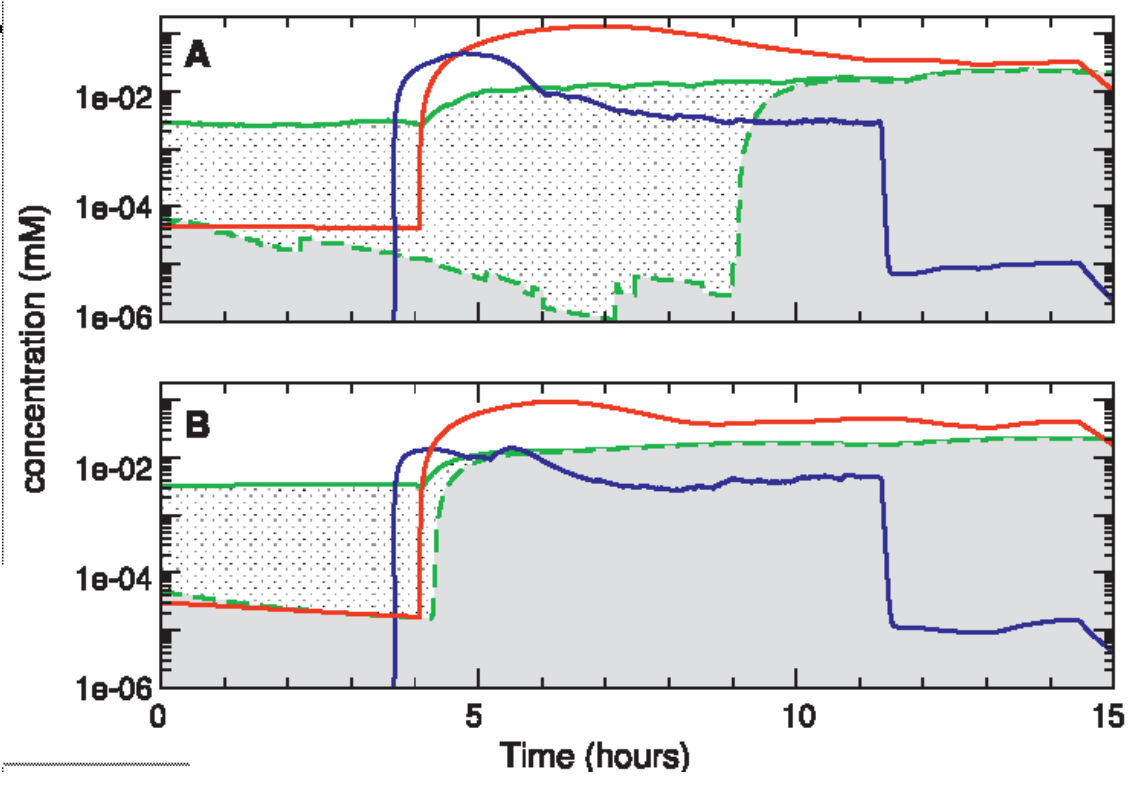
black: internal protein number

evolution of lac operon with stoch. prot. expression avoids bistability even more



dotted: start (bistable); solid evolved stoch.; dashed evolved det.

WHY?
long delay in induction in stoch model
when in bistable regime (i.e. low repressed expression)



A: stochastic; B: deterministic

red ext. lac; blue ext. gluc; green βGalactosidase solid line: at high γ dotted at low γ

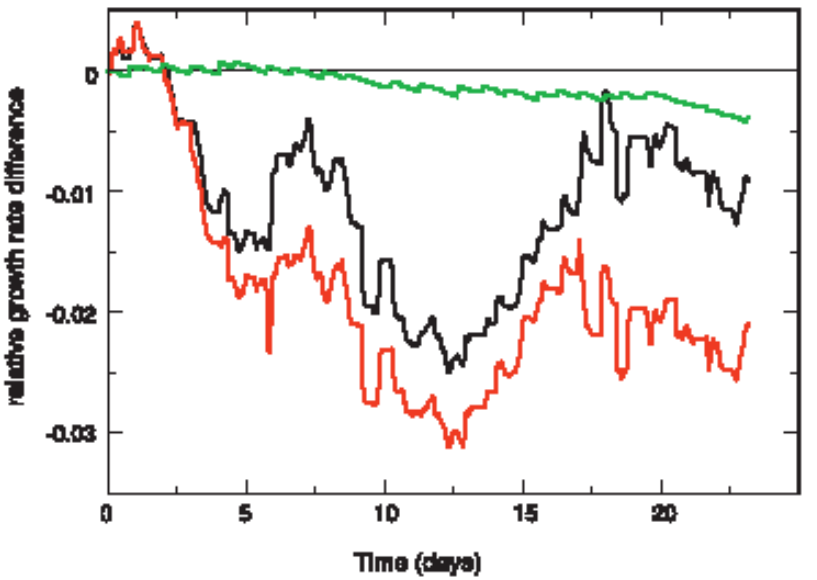
Relative Growth rates of promotor functions in deterministic and stochastice models

all times resource pulses are different
low repressed expression 'better' when no lactose and vv
therefor compare growth-rates over time relative to deterministic, low repressed rates

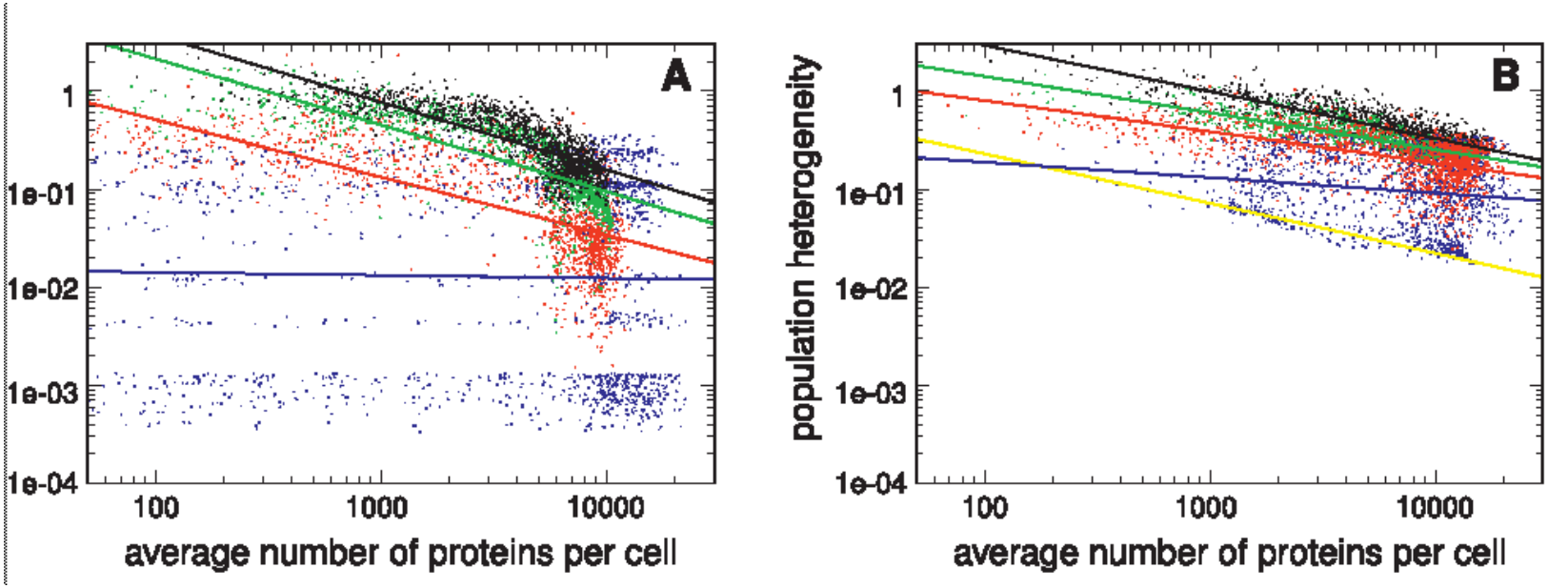
green: deterministic, high repressed

black stoch. : high repressed rates

red stoch: low repressed rates



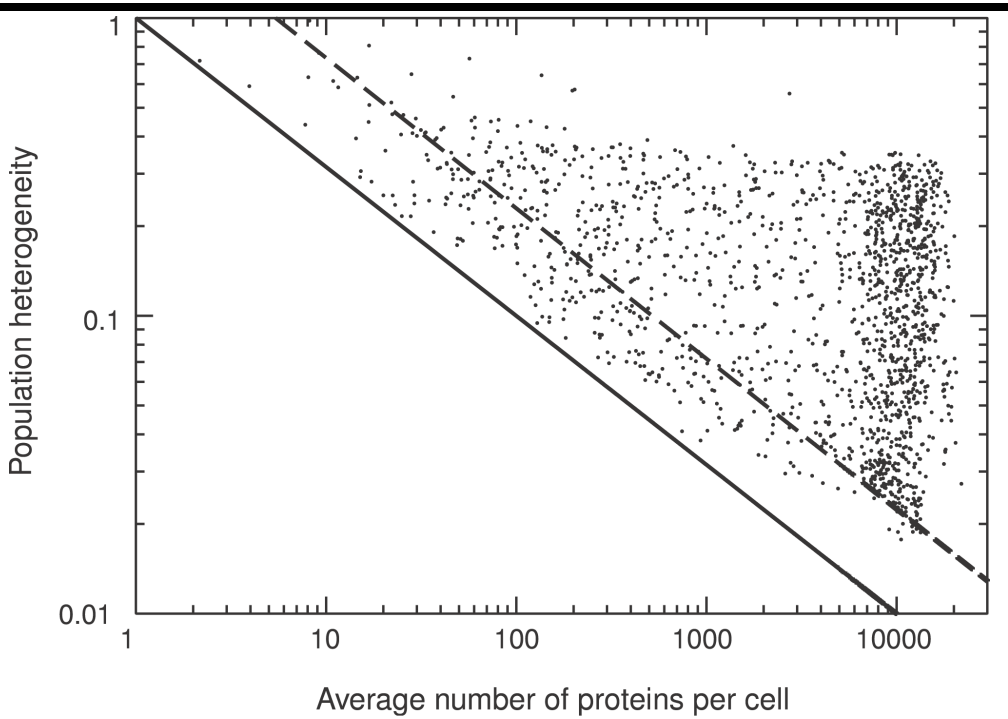
population heterogeneity in various model variants: deterministic vs stochastic; genetic vs one clone, spatial vs well mixed



deterministic stochastic

black: full model; red: well mixed; green 1 clone full model; blue 1 clone well mixed; note partial synchronization; yellow intrisic noise

Population heterogeneity can be smaller than intrinsic noise because of non-equillibrium circumstances



(during decay of proteins no heterogeneous burstsizes)

conclusions

Bistability even more detrimental when stochasticity is taken into account

on induction: long waiting for large bursts.

role of stochasticity overestimated by considering genetically identical cells in a homogeneous environment in equilibrium

non-equilibrium conditions can reduce population heterogeneity

large genetic heterogeneity in natural populations: fast adaptation to environmental condition

interlocking of evolutionary and regulatory timescales!

Parameter uncertainty inherent in evolution

Experimental and Modeling strategies

Experiments: use 'controlled conditions'

Mini-models: can study parameter space and 'choose' parameters based on outcome (fitting experiments)

Detailed models: use (MANY) measured / estimated ('reasonable') parameters

minimal evolutionary optimization models ('what is it good for?) (bet-hedging)

Here use multilevel (evolutionary) modeling to generate parameters and debug the above