

Metabolic regulation
Using Evolution to understand genome
structure and transcription regulation

Present day (evolved) metabolism studied from evolutionary perspective

Last time:

By assuming optimality and equilibrium 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 evolutionary perspective

Lac operon

causes of diabetes ("causal drift")

"regulation of mutations" (TOR pathway; ribosomal RNA)

Experimental and Modeling strategies

Experiments: use 'controlled conditions'

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

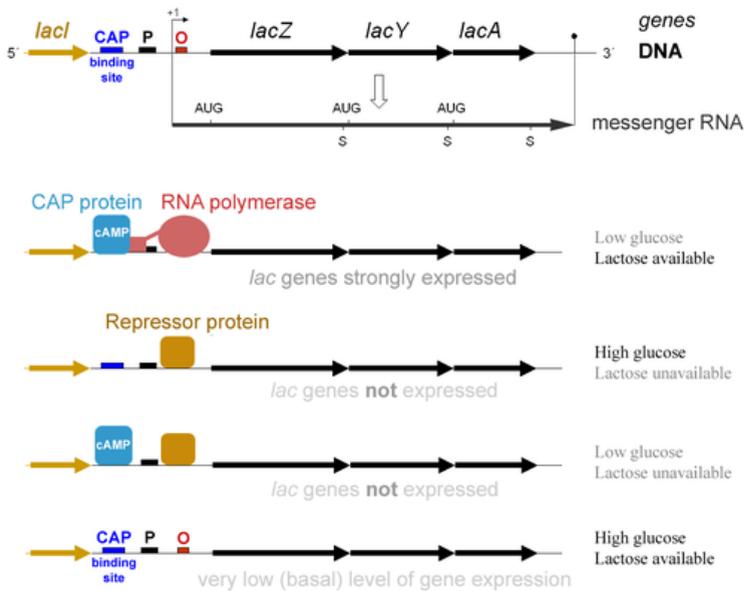
Detailed models: use (MANY) measured / estimated ('reasonable') parameters

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

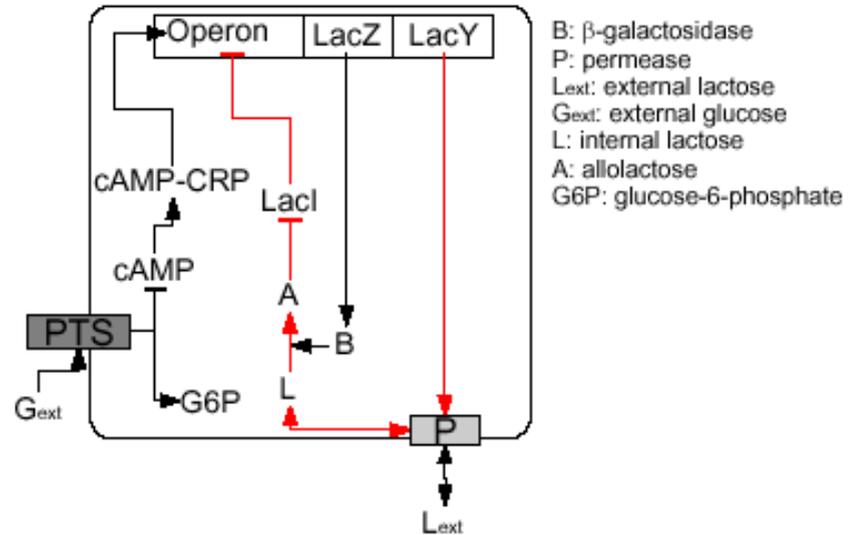
*Here use multilevel (evolutionary) modeling
to generate parameters and debug the above*

Prototype gene regulation: Lac operon

The *lac* Operon and its Control Elements



An overview of the *lac* operon



genome structure

regulatory network

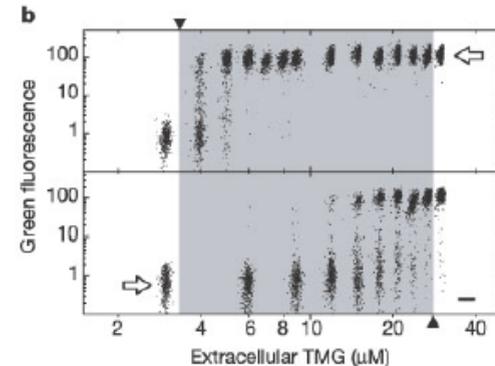
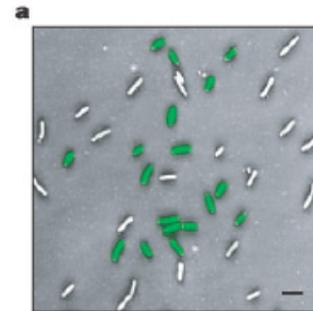
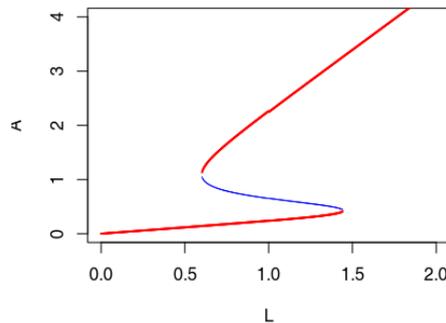
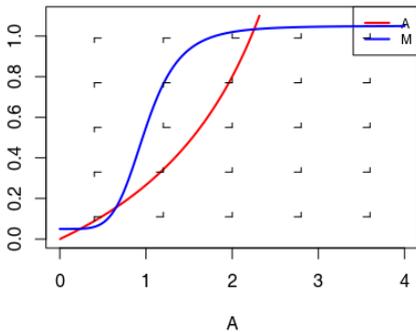
Lac Operon: Prototype bi-stability in gene regulation: classical mini-model, experiments

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

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

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

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



bi-stability

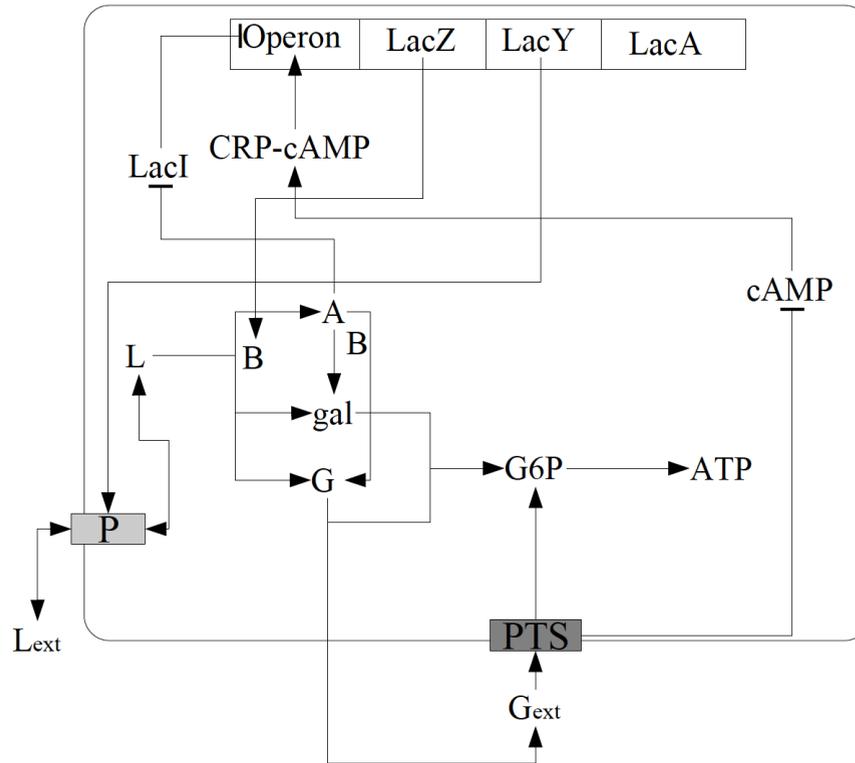
experimentally “verified”

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

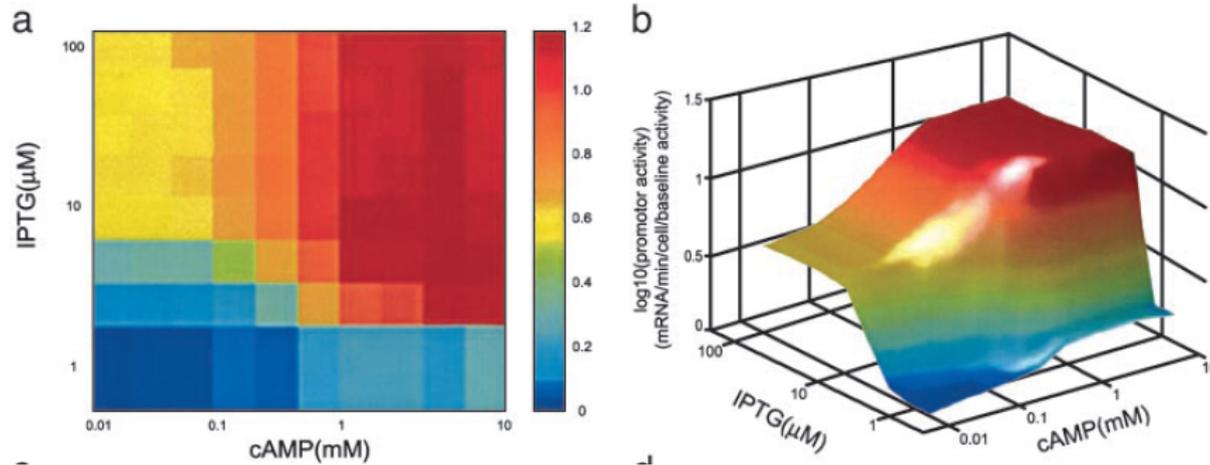
Metabolic regulation in E.coli

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

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

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.

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

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

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

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

$V_1 \dots V_5$ 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.

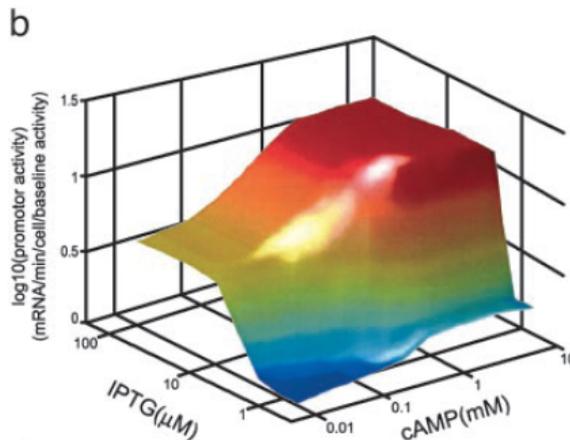
$c = LACI_T/k_{LACI}$, the total LacI concentration in units of its dissociation constant for binding to its site.

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

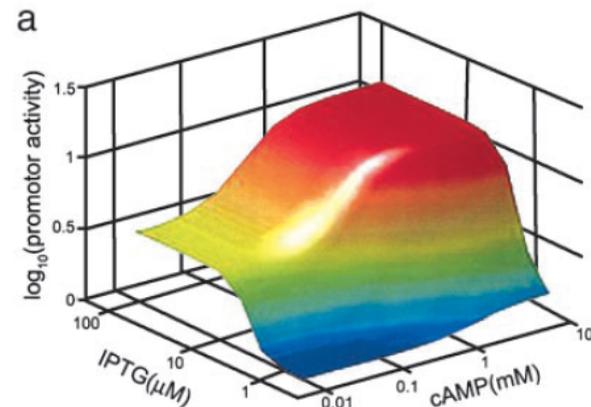
α , the transcription rate when RNA Polymerase is bound to the DNA, but CRP and LACI are not.

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)

~ ND

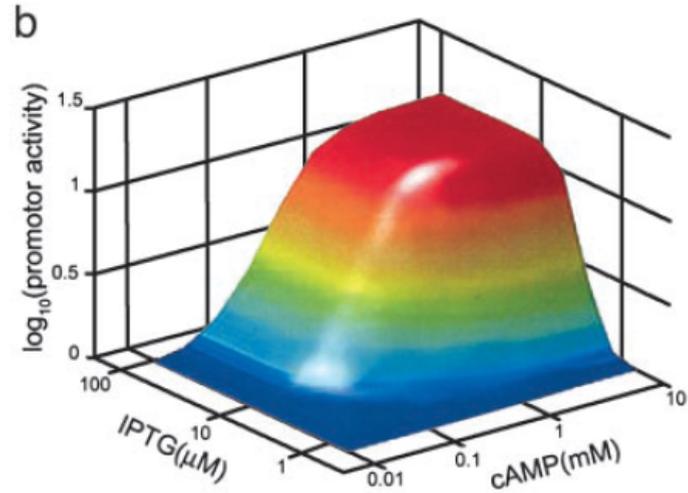
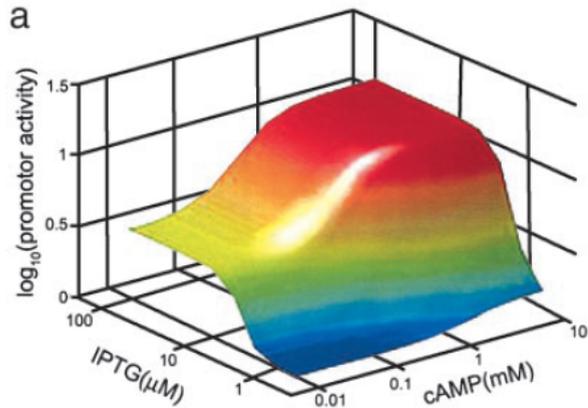
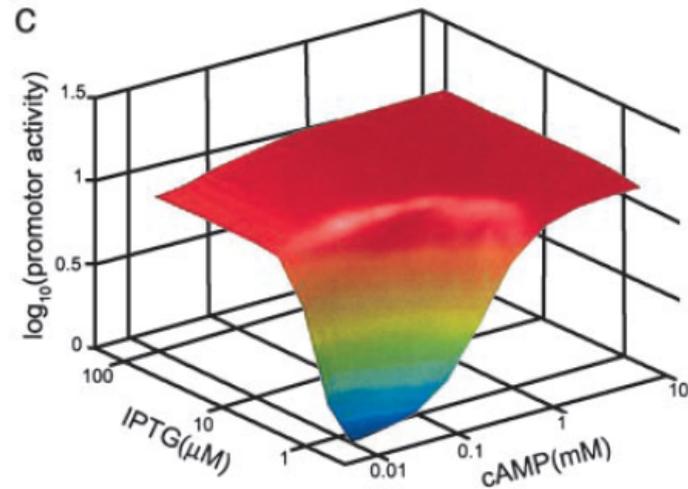


Table 1. *lac* model parameters that best fit the measurement using the GFP reporter plasmid (wild type) and putative mutants that have purer AND-like and OR-like gates

Parameter	Wild type	AND	OR
m	4 ± 0.6	4	4
n	2 ± 0.4	2	2
$K_{IPTG}, \mu M$	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



OR

more complex model of the lac operon

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

$$PA(A, C) \equiv V_1 \frac{1 + \frac{V_2(C/k_C)^n}{1+(C/k_C)^n} + \frac{V_3}{1+(A/k_A)^m}}{1 + \frac{V_4(C/k_C)^n}{1+(C/k_C)^n} + \frac{V_5}{1+(A/k_A)^m}}$$

$$\frac{dM}{dt} = P(A, C) - (\gamma_M + \mu)M$$

$$\frac{dB}{dt} = k_B M - (\gamma_B + \mu)B$$

$$\frac{dP}{dt} = k_P M - (\gamma_P + \mu)P$$

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)

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

Wong concluded : bistable switch

Table 1: All model parameters with their values.

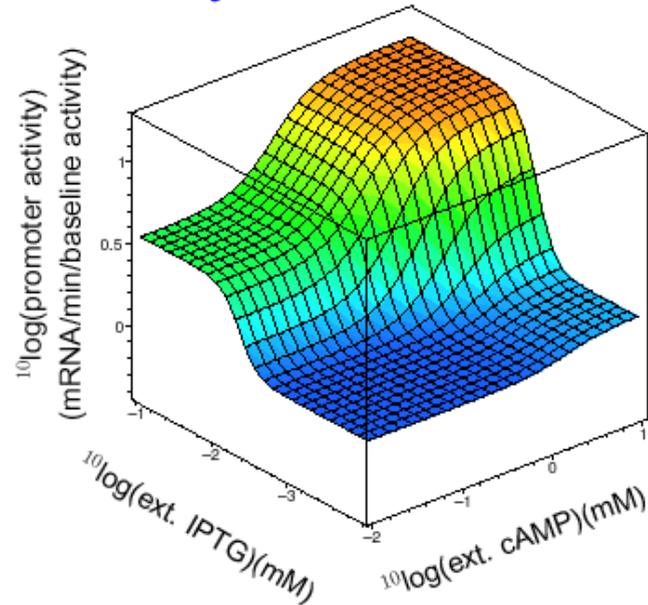
parameter	equation	value	comments
k_C	Eq. 2	evolvable, mM	initial value: 1.0×10^{-3} mM
n	Eq. 2	evolvable	initial value: 4.0
k_A	Eq. 3	evolvable, mM	initial value: 5.5×10^{-4} mM
m	Eq. 3	evolvable	initial value: 8.0
a	Eq. 4	evolvable	initial value: 1.0
b	Eq. 4	evolvable	initial value: 1.0
c	Eq. 4	evolvable	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	0.093/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	0.093/min	fitted with data of Hogema et al. (6)
k_t, Glu	Eq. 15	45 mM/min	Wong et al. (2), Carlson and Srien (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 Srien (11)
GC	Eq. 22	7.28×10^5 mM	estimated with data of Carlson and Srien (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_{maz}	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
$V_{mRNA,maz}$		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)!

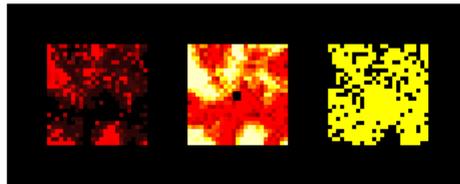


Setty Alon 2003

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

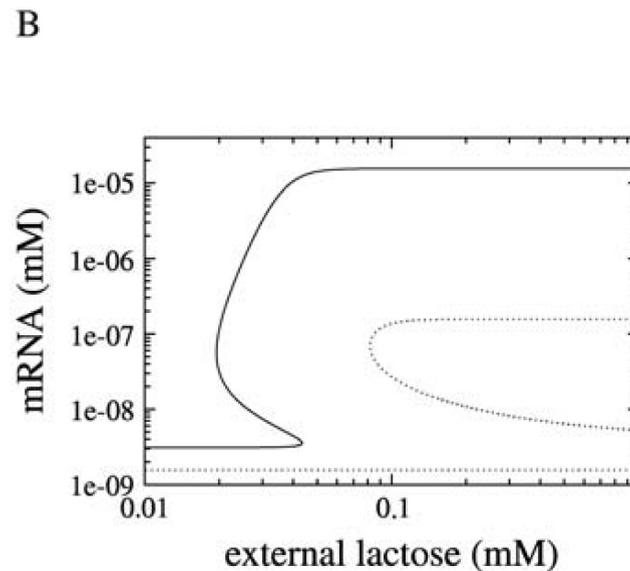
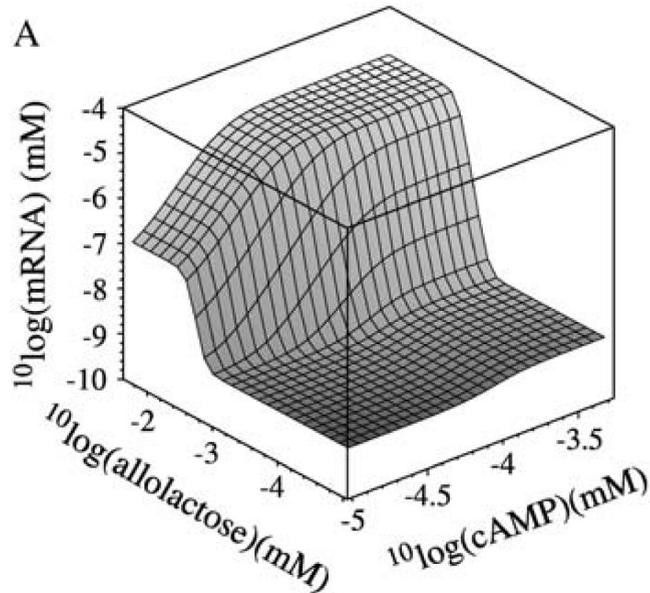
**“experimental setup” evolution of the Lac operon:
timescales: metabolism, cell growth/division, prot. stab,
environmental switches, evolution**

- 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 ($2 \times$ size), decay (density dep; no ATP)
- encountered environments depend on dynamics! dynamics!



evolution as trick to cope with parameter uncertainty

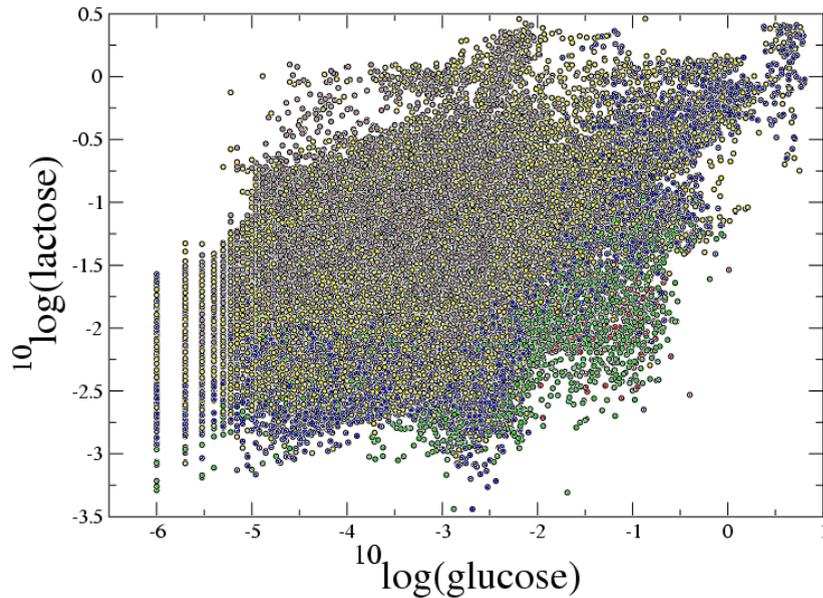
initialize as a bistable switch
(because no bistable switch evolved...)



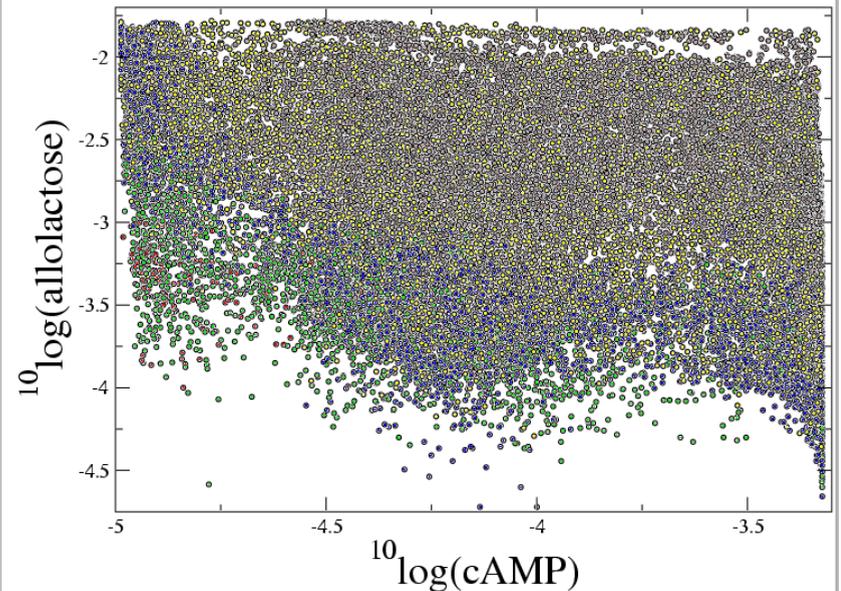
solid low glucose; dotted high glucose

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

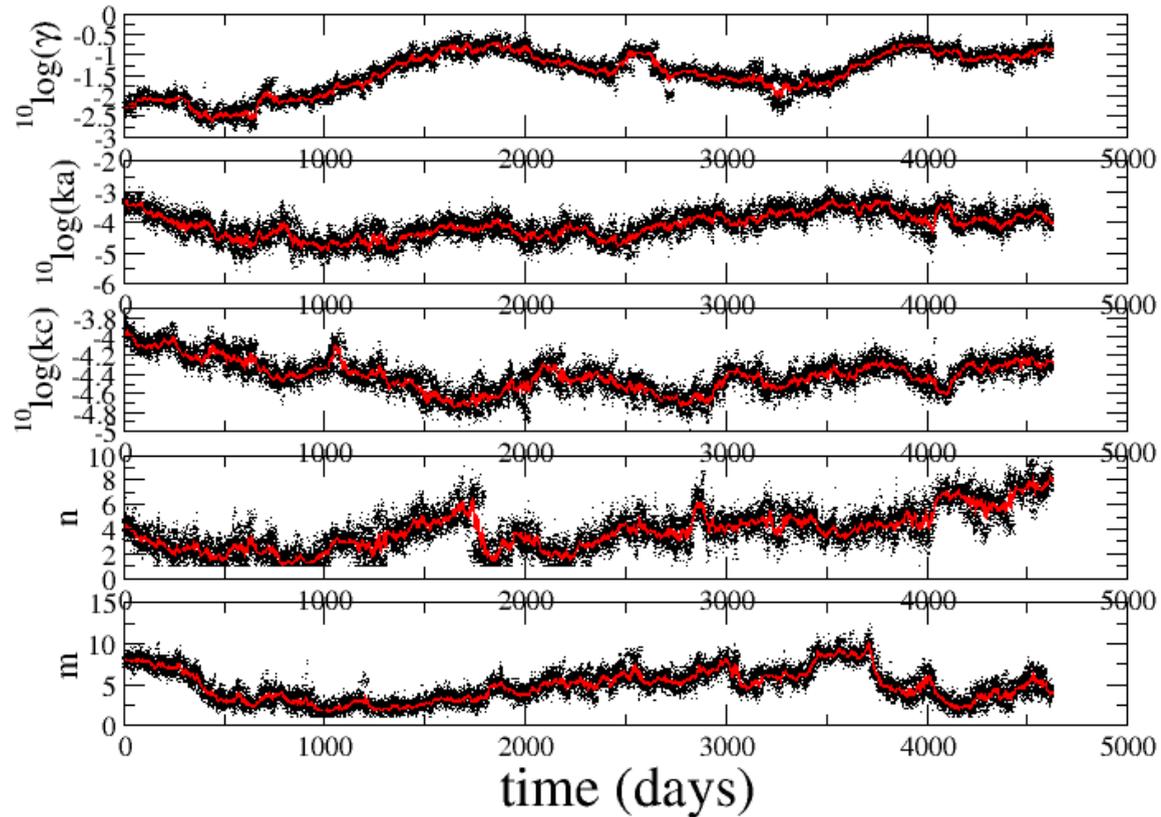
Glucose vs. lactose, at end of evolution



cAMP vs. allolactose, at end of evolution

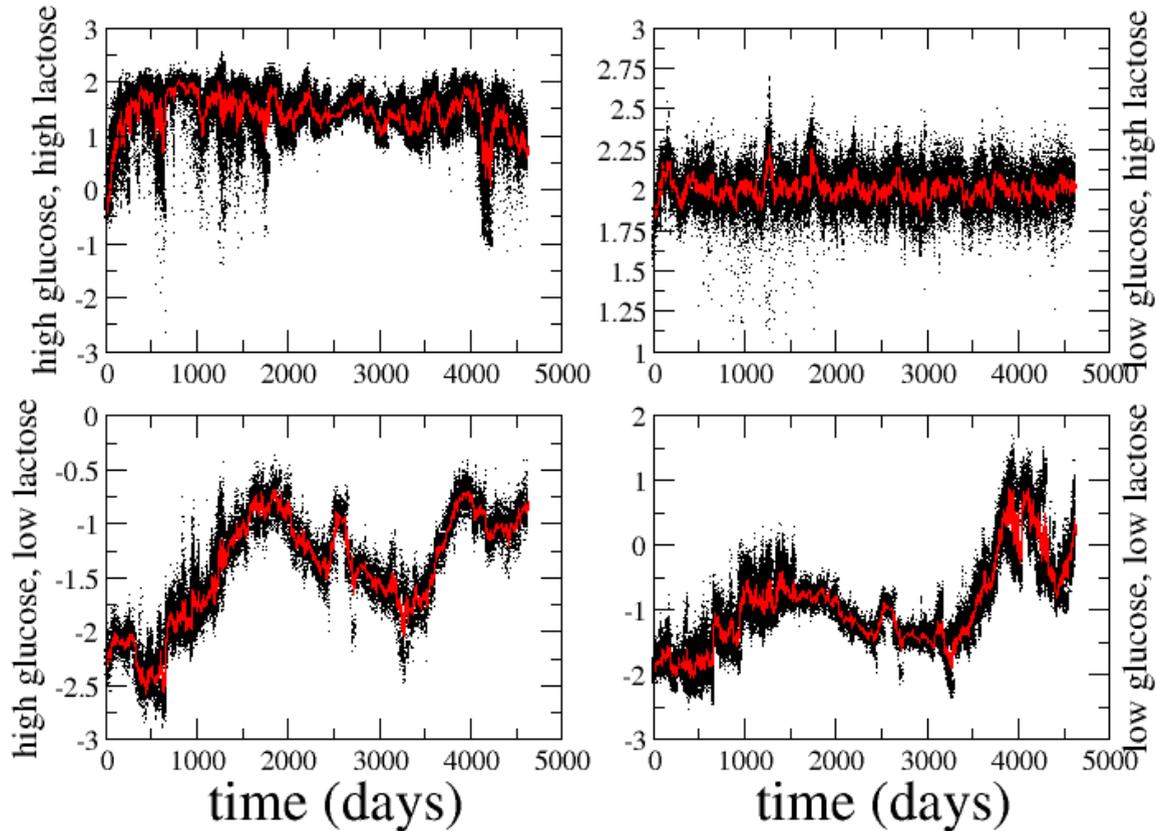


Evolution: how to observe parameter of individuals in pop. in time



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

Evolution of 4 corners



Evolution: how to observe comparison of evolutionary outcomes

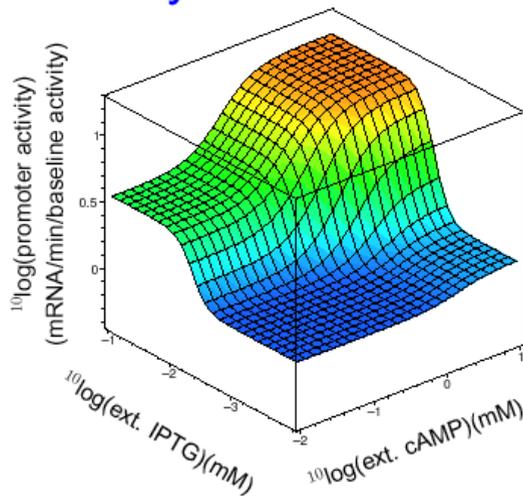
Ancestor trace!

Compete last common ancestors (n^*)

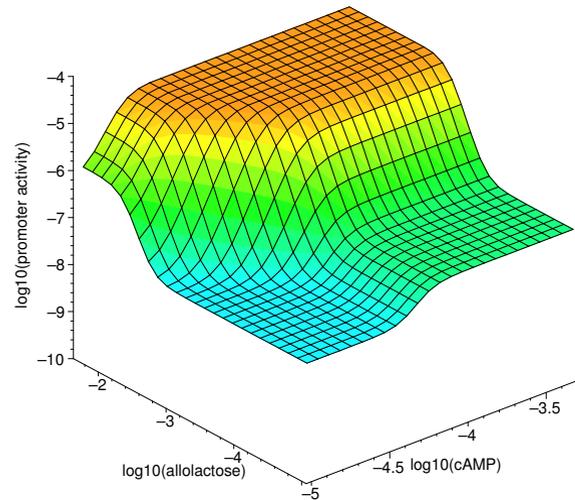
Compete last populations (n^*)

-- > "BEST" evolved promoter function

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

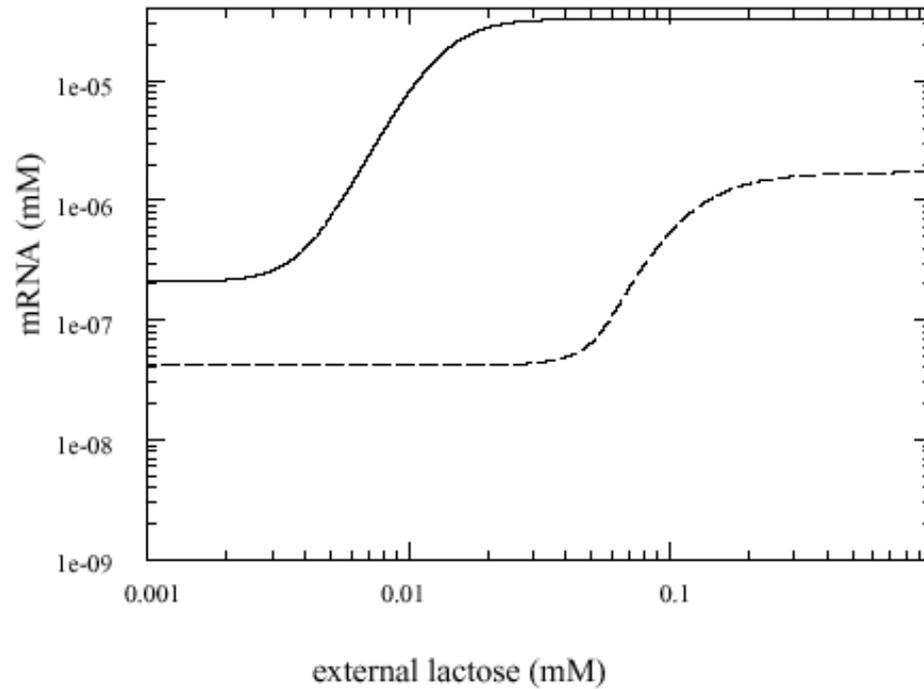


OBSERVED (Setty 2003)



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.

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

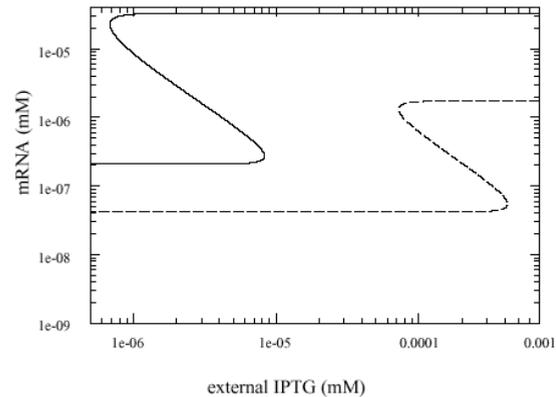
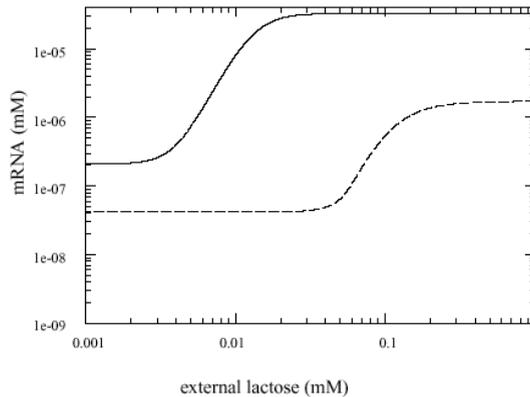
for lactose:

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

for artificial inducer

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

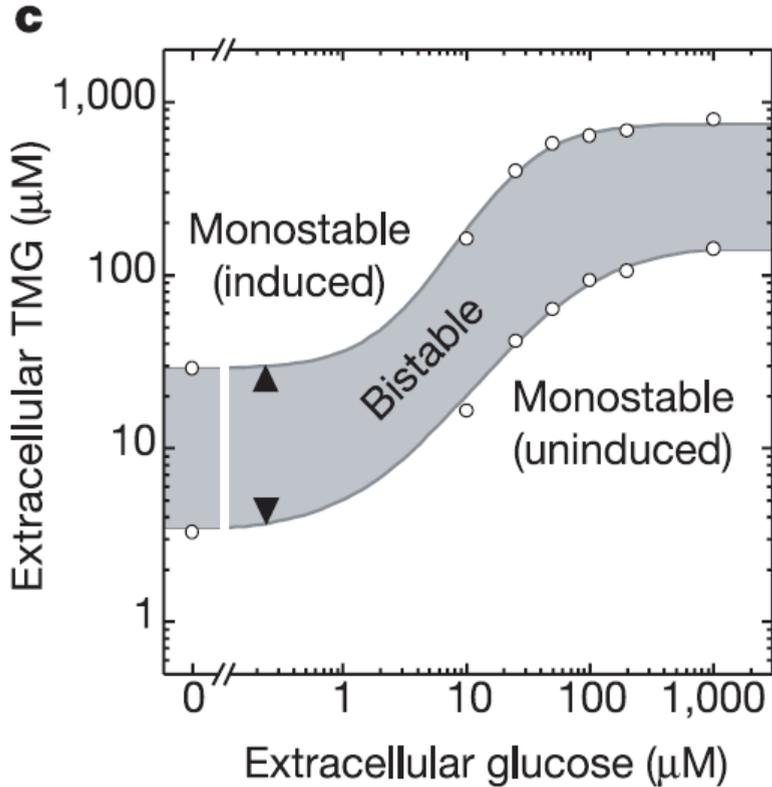
Evolved promoter function bistable for artificial inducer!



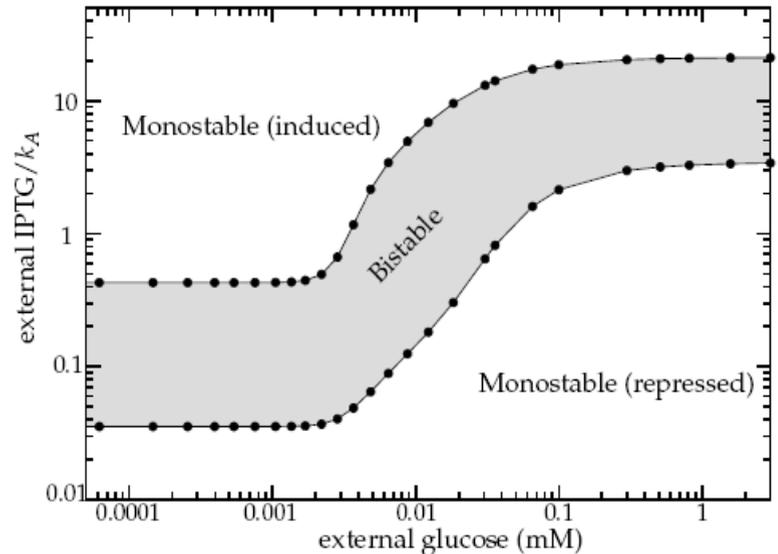
LACTOSE

ART INDUCER

evolved vs measured bistability for artificial inducer

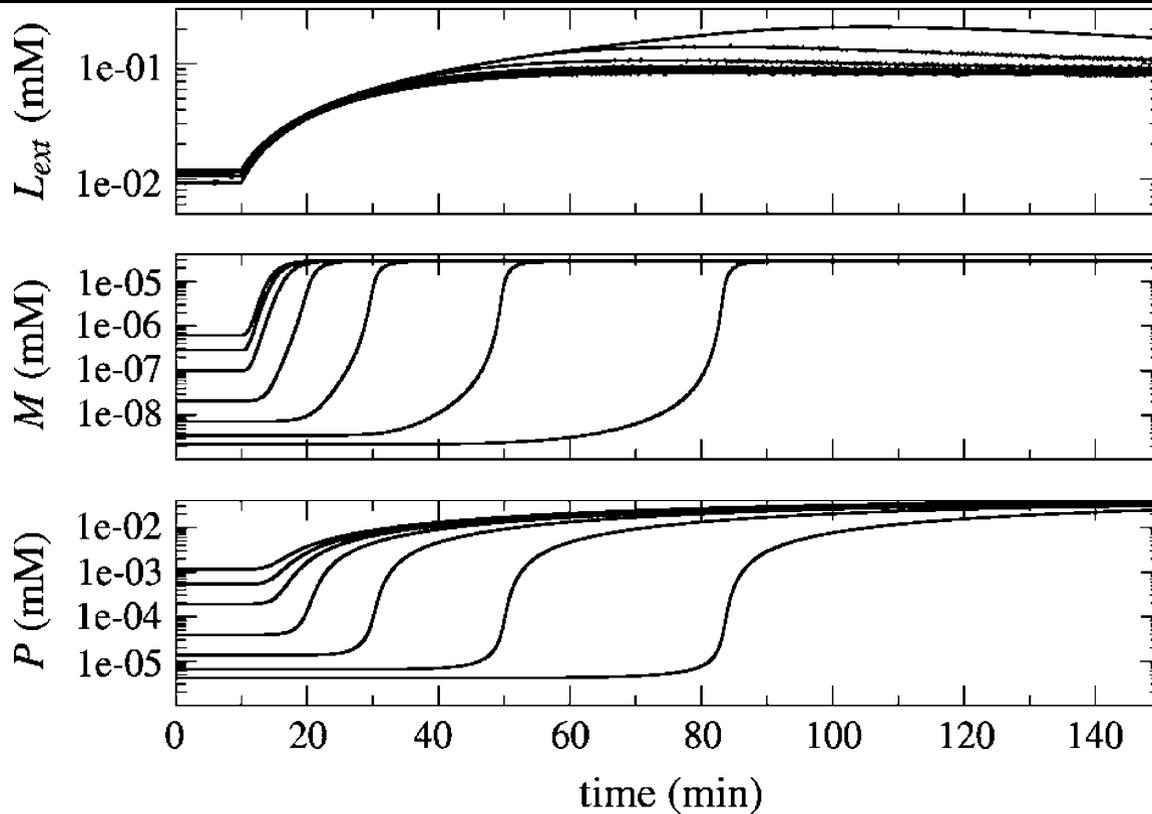


measured (van Oudenaarden)



evolve (van Hoek)

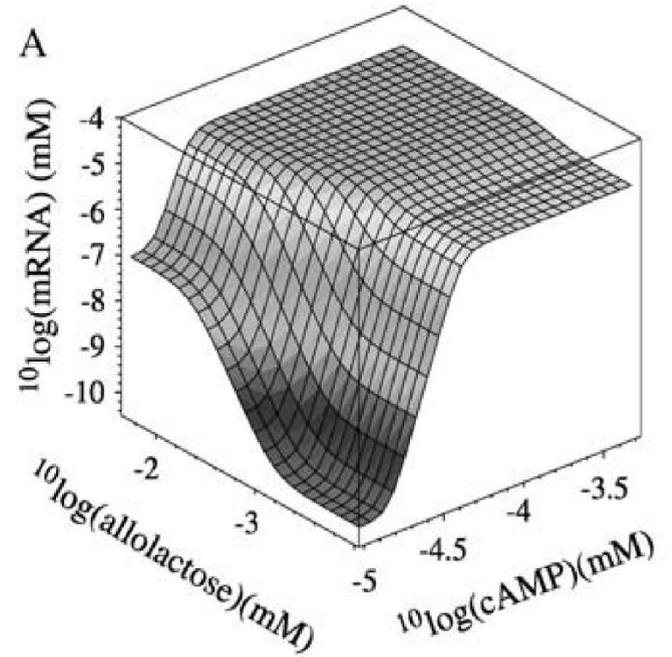
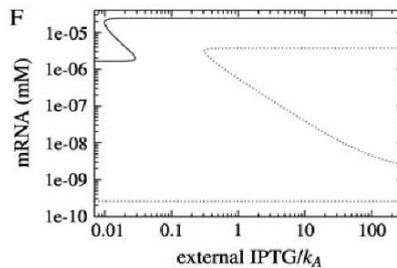
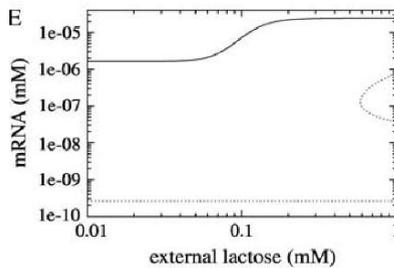
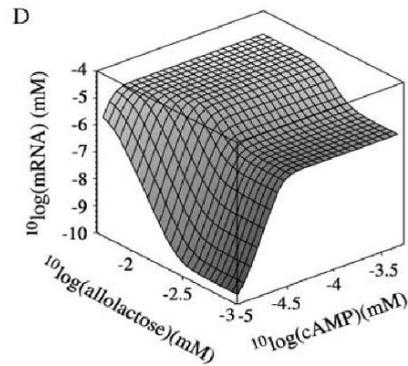
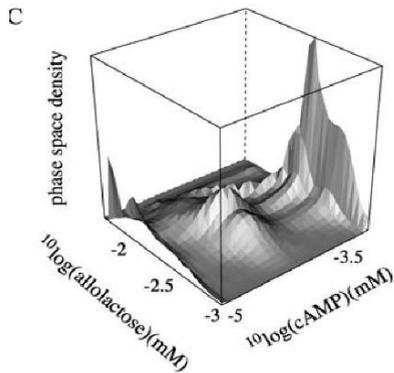
Why avoid bistability
why 'waste' expression when no (low) Lactose available
Non-equilibrium: delays!



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

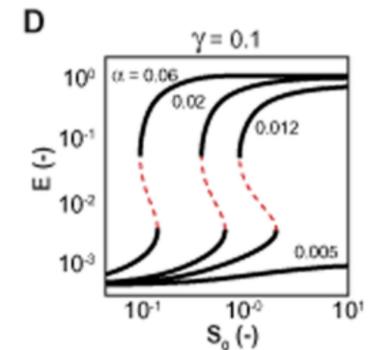
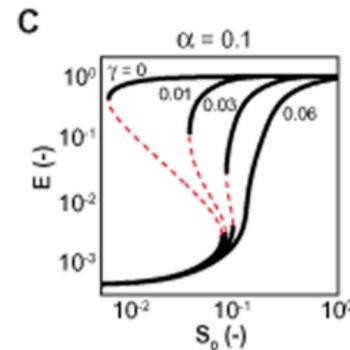
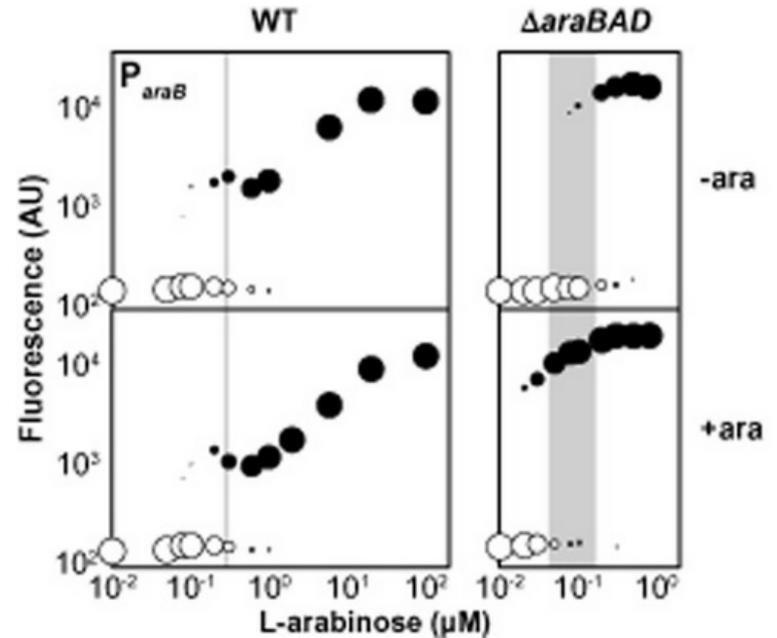
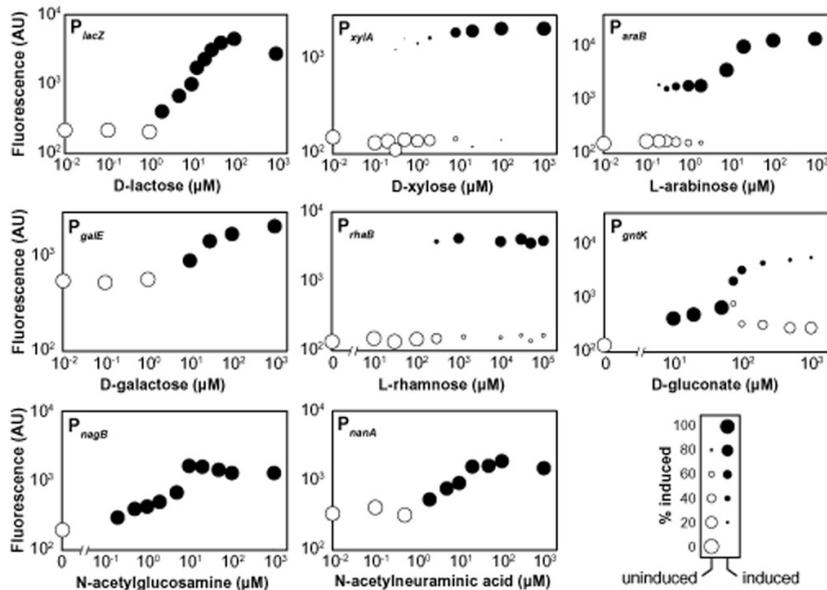
E.M. Ozbudak, M. Thattai, H.N. Lim, B.I. Shraiman, A. Van Oudenaarden Multistability in the lactose utilization network of *Escherichia coli*. *Nature*, 427 (2004), pp. 737

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.**

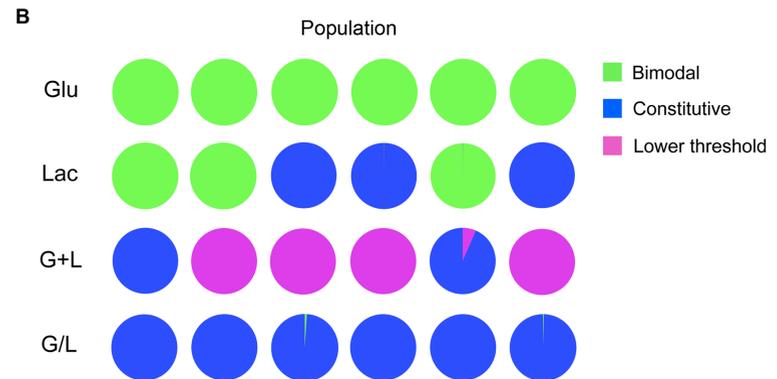
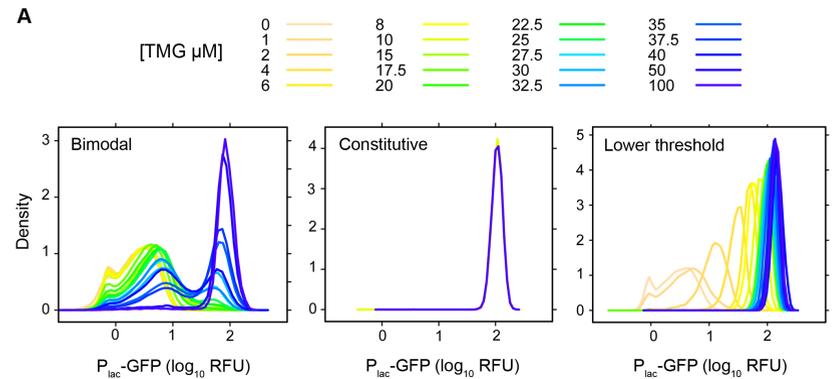
various responses for different sugars

suppressing catabolism enhances hysteresis



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



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)

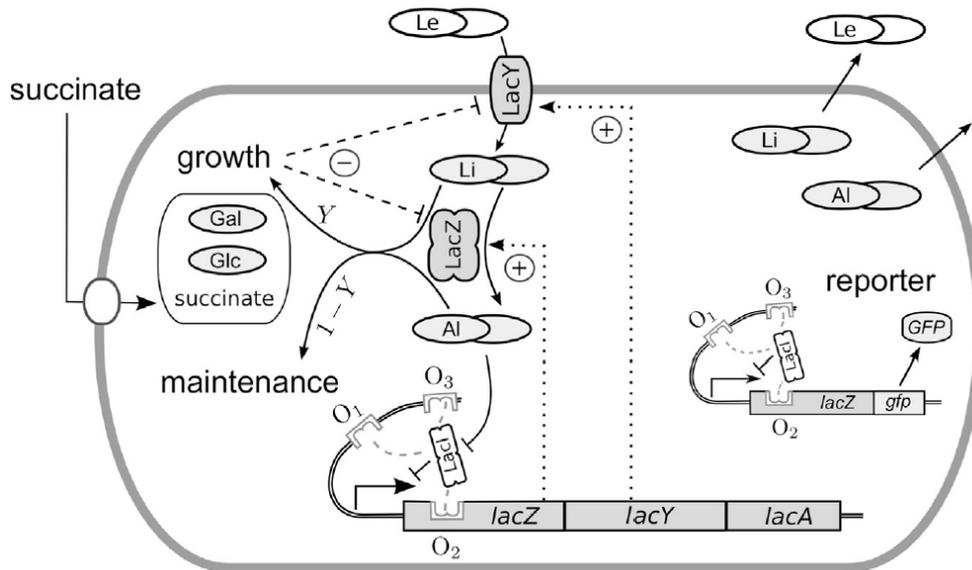
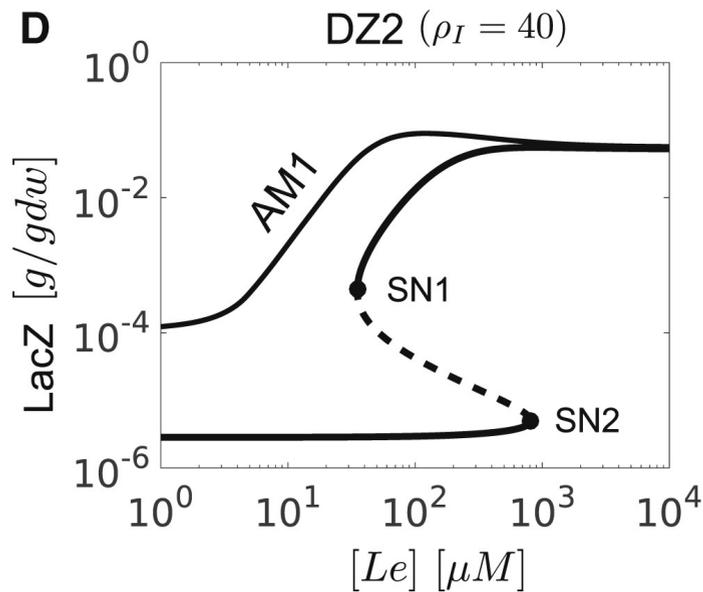
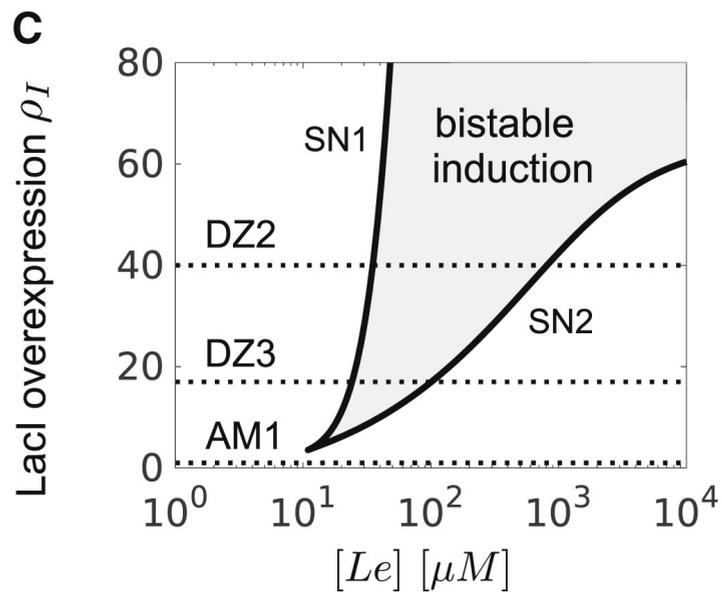
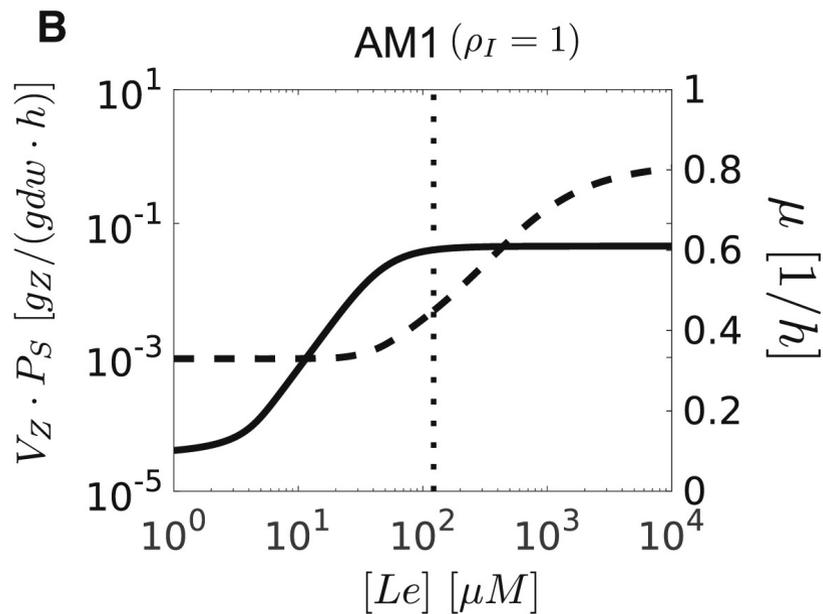
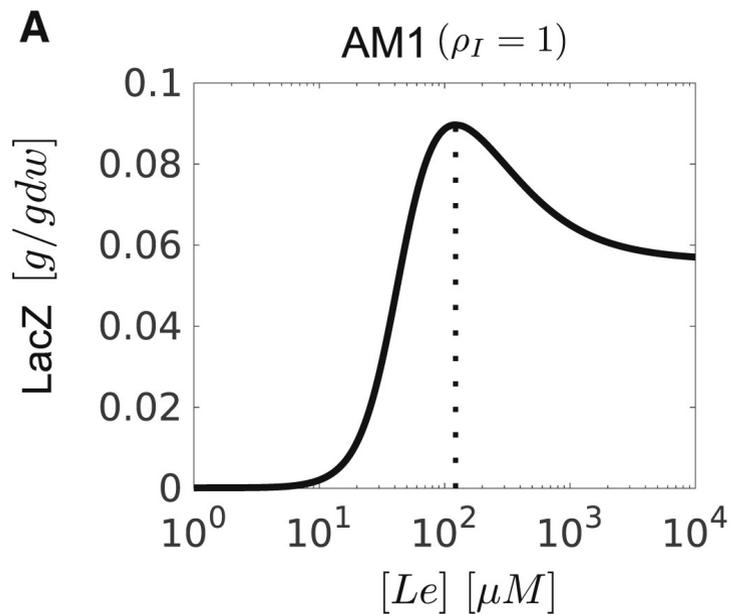


TABLE 2 Model Parameters

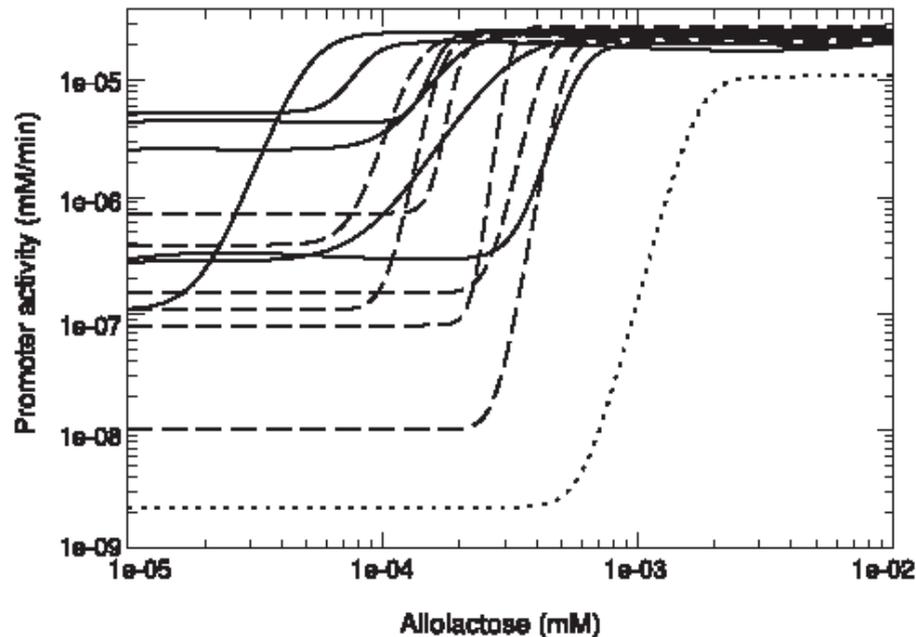
Name	Value	Reference	Name	Value	Reference
V_L	$1271 \frac{gL/gdw}{gy/gdw} \frac{1}{h}$	(20)	κ_2	0.38	(34)
K_L	$0.68 \frac{gL}{L} (\approx 2 \text{ mM})$	estimated	α_1	31	(34)
K_i	$0.00013 \frac{gL}{gdw}$	estimated	$\hat{\alpha}_1$	1420	(34)
V_{lac}	$670 \frac{gL/gdw}{gz/gdw} \frac{1}{h}$	(30)	α_2	19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



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 than 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 heterogeneity.

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

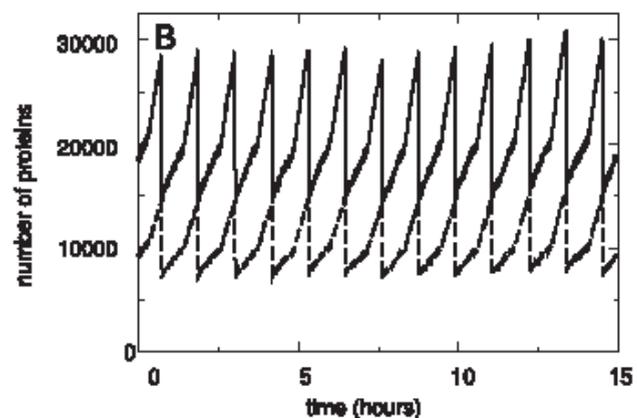
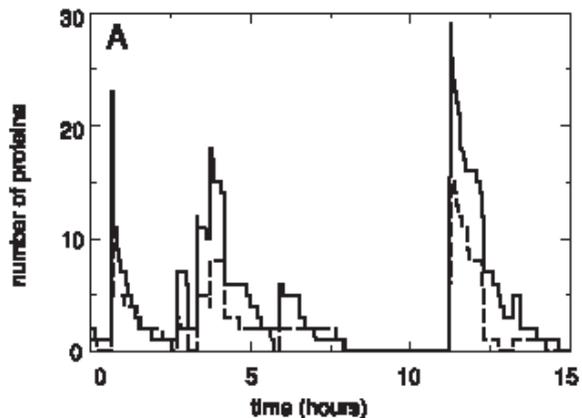
But: Excessive stochasticity of promoter function measured in E. coli (Wolf & van Nimwegen 2016)

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

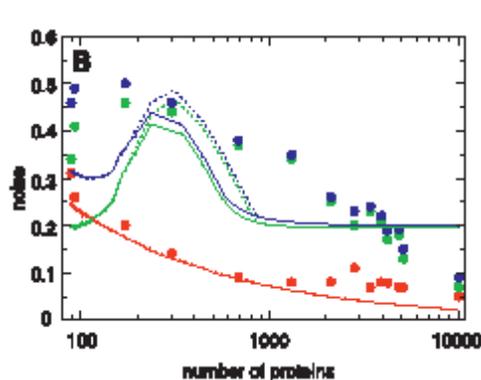


intrinsic vs extrinsic noise: experiments and model

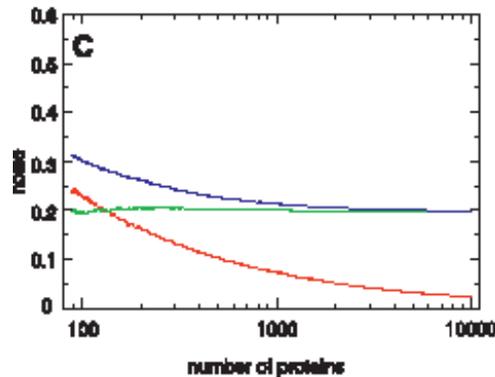
extrinsic noise: cell cycle + intracellular inducer concentration (green)

intrinsic noise: difference in expression of 2 identical promoters in a single cell (red)

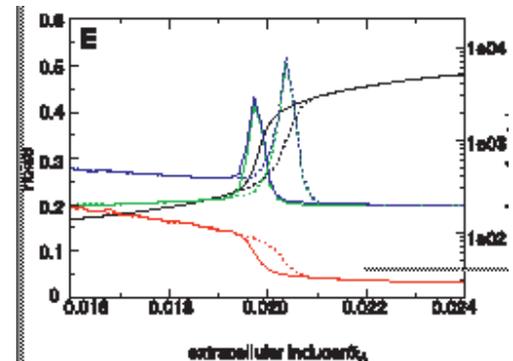
$N_{tot} = N_{ext} + N_{int} = std/mean$ in population (blue)



IPTG as inducer



Lac as inducer

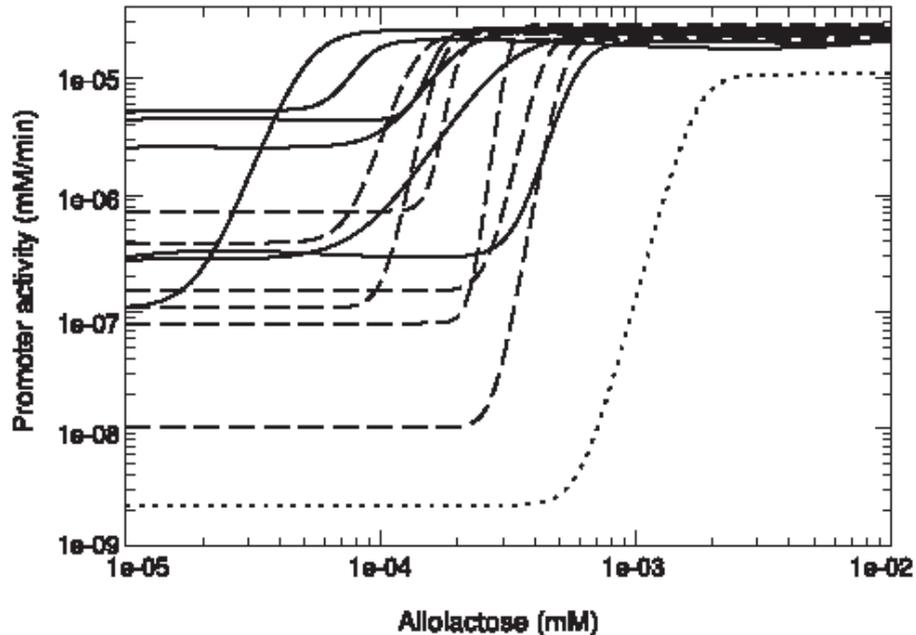


IPTG as inducer

noise relative to internal protein numbers relative to external IPTG

black: internal protein number

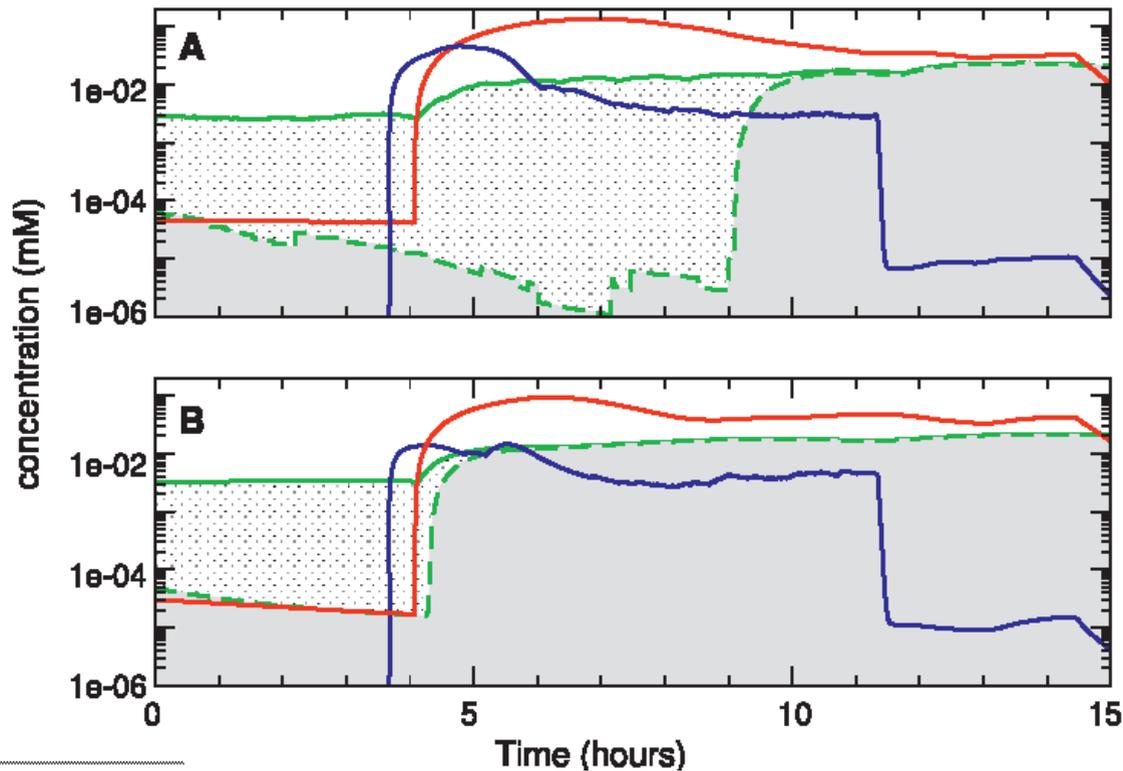
evolution of lac operon with stoch. prot. expression avoids bistability even more



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 β Galactosidase solid line: at high γ dotted at low γ

Relative Growth rates of promotor functions in deterministic and stochastic models

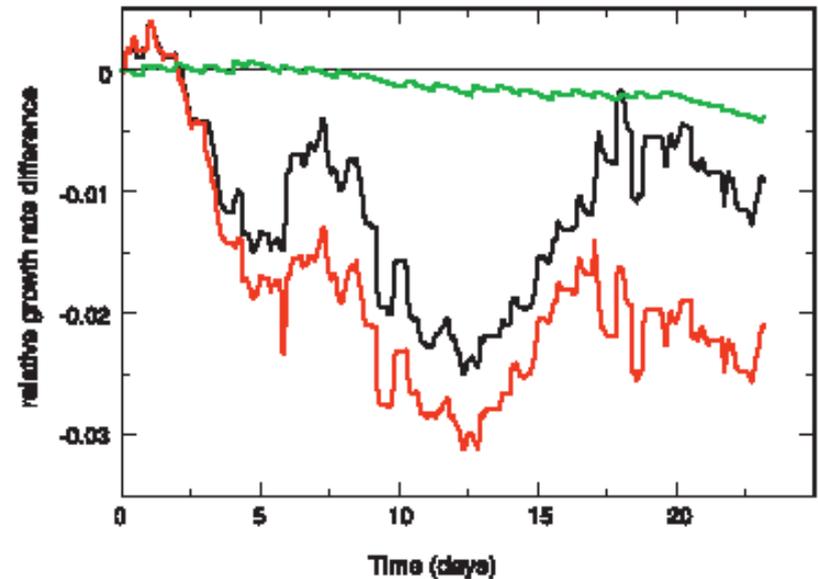
all times resource pulses are different

low repressed expression 'better' when no lactose and vv therefor compare growth-rates over time relative to deterministic, low repressed rates

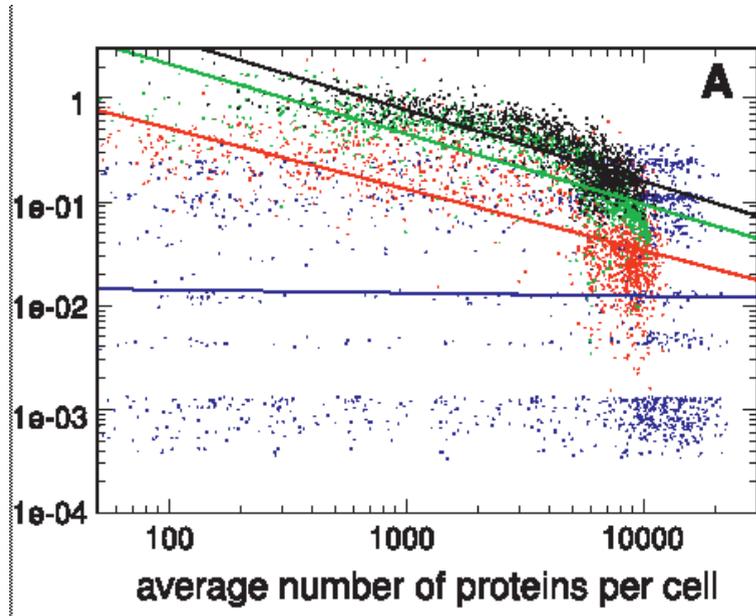
green: deterministic, high repressed

black stoch. : high repressed rates

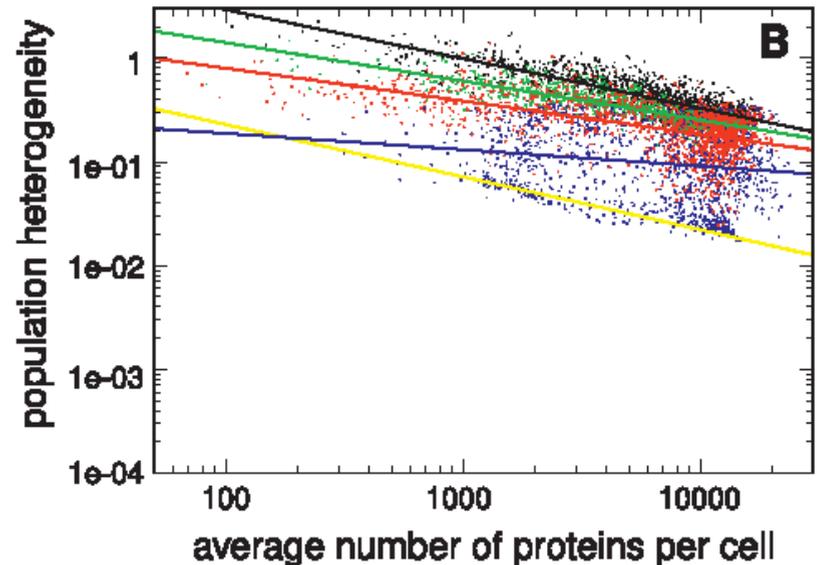
red stoch: low repressed rates



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



deterministic

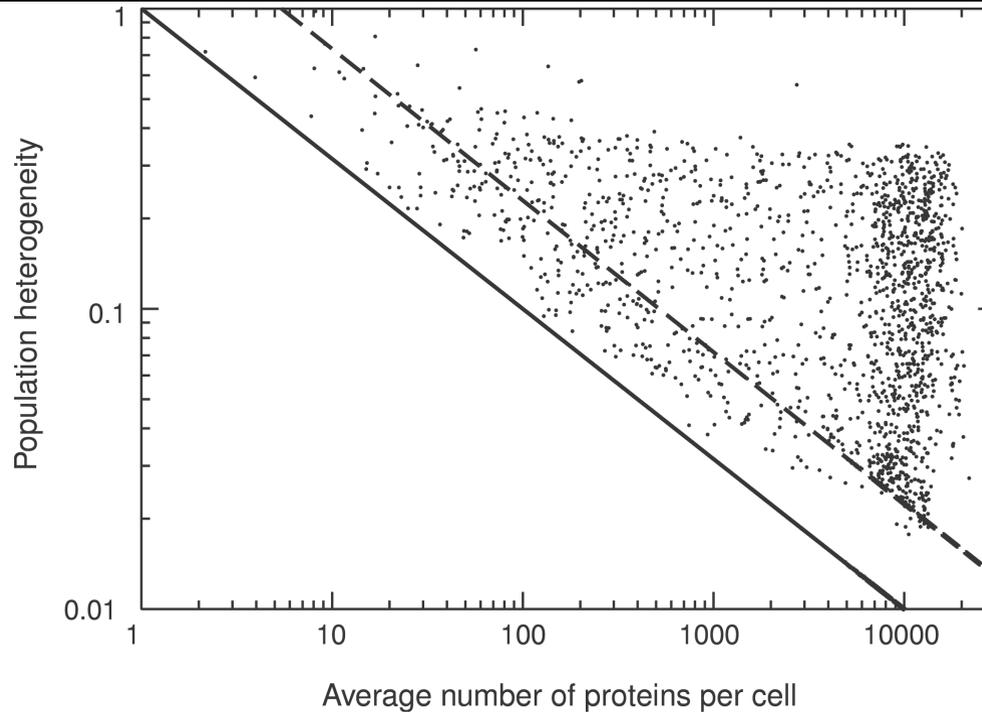


stochastic

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

Model	Source of Heterogeneity	Regression	Correlation
Stochastic	Black: spatial + genetic	-0.47	-0.85
	Green: spatial	-0.37	-0.86
	Red: genetic	-0.32	-0.58
	Blue: no	-0.16	-0.16
	Yellow: intrinsic noise	-0.51	-1.00
Deterministic	Black: spatial + genetic	-0.68	-0.81
	Green: spatial	-0.67	-0.78
	Red: genetic	-0.59	-0.71
	Blue: no	-0.029	-0.025

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



(during decay of proteins no heterogeneous burst sizes)

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)

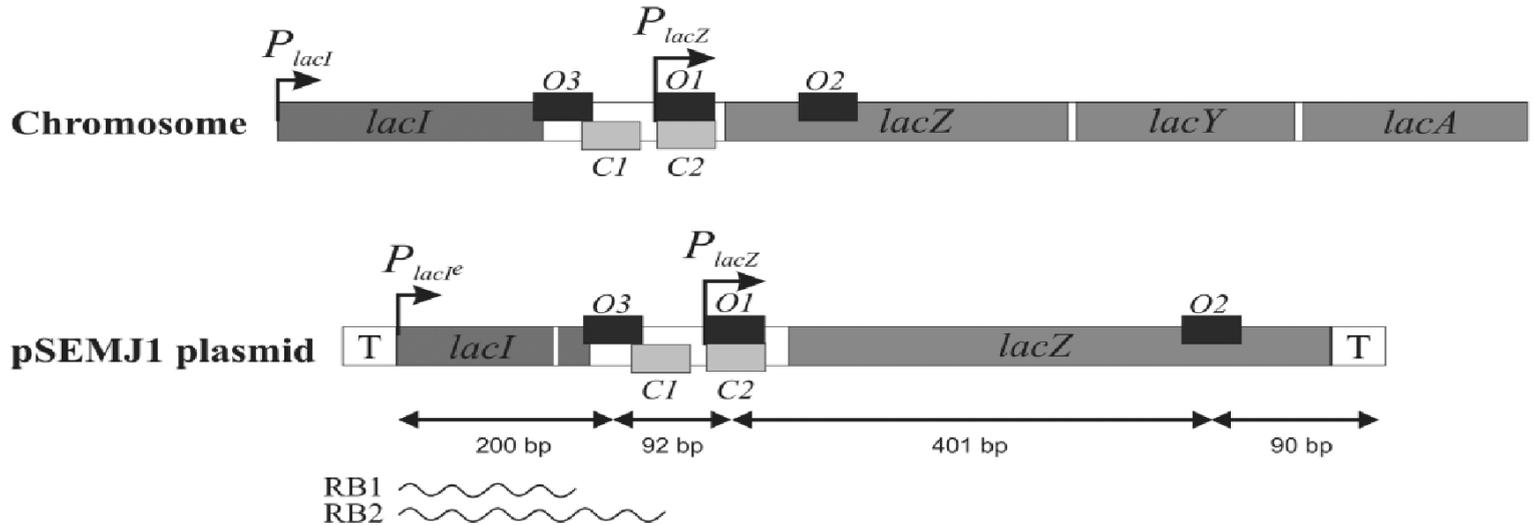
Detailed models: use (MANY) measured / estimated ('reasonable') parameters

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

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

MOREOVER: LACI autoregulator!

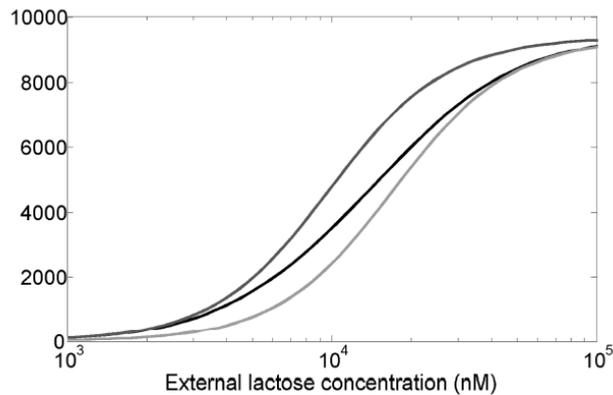
Semsey et al 2013



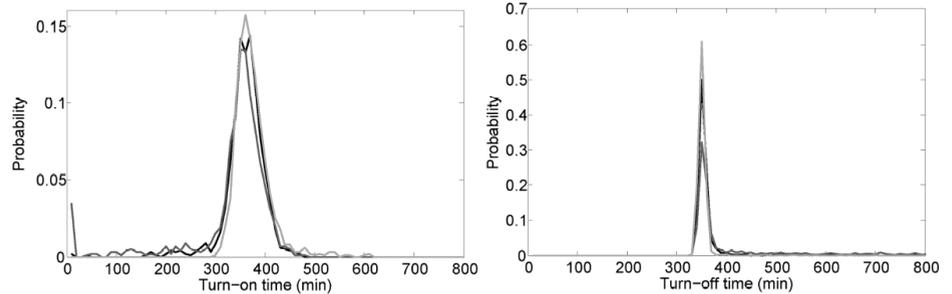
Binding state	Relative probability	LacI production	LacZYA production
unbound	1	1	1
$O1$ bound	$\epsilon_1 I^*$	1	0
$O1$ - $O2$ loop	$\epsilon_2 I^*$	1	0
$O1$ - $O3$ loop	$\epsilon_3 I^*$	0	0

LacI autoregulation leads to smoother activation, and less variation in delays relative to constitutive expression.

LacY expression



Delay in expression after switch



deterministic model

stochastic model

Table 2. Mean \pm standard deviations of turn-on and turn-off times obtained in the simulations shown in Figure 6

	LacI range: 30–90 molecules			LacI range: 10–30 molecules		
	WT (autoregulated)	Constant low	Constant high	WT (autoregulated)	Constant low	Constant high
Turn-on time (minutes)	354.2 \pm 41.7	330.5 \pm 85.4	368.8 \pm 36.0	349.3 \pm 44.2	308.7 \pm 103.7	364.8 \pm 38.3
Turn-off time (minutes)	363.7 \pm 74.4	468.9 \pm 246.0	348.4 \pm 33.0	394.0 \pm 128.6	602.0 \pm 392.5	354.4 \pm 50.5