Stochastic simulations

Application to molecular networks

Literature overview

Noise in Gene Expression: Origins, Consequences, and Control

Jonathan M. Raser[1,2] and Erin K. O'Shea[2*†]

Genetically identical cells and organisms exhibit remarkable diversity even when they have identical histories of environmental exposure. Noise, or variation, in the process of gene expression may contribute to this phenotypic variability. Recent studies suggest that this noise has multiple sources, including the stochastic or inherently random nature of the biochemical reactions of gene expression. In this review, we summarize noise terminology and comment on recent investigations into the sources, consequences, and control of noise in gene expression.

Any individual in a population of living organisms or cells is unique. Much of population variability is due to genetic differences, but environment and history also contribute to variability in cellular phenotype. Indeed, identical twin humans or cloned cats differ in appearance and behavior (Fig. 1). However, even cells or organisms with the same genes, in the same environment, with the same history, display variations in form and behavior that can be subtle or dramatic. Investigations have focused on the possibility that such variability is inevitable in biological systems because of the random nature of chemical reactions within a cell (*1*). When large numbers of molecules are present, chemical reactions may proceed in a predictable manner. However, when only a few molecules of a specific type exist in a cell, stochastic effects can become prominent.

Gene expression, as defined by the set of reactions that control the abundance of gene products, influences most aspects of cellular behavior, and its variation is often invoked to explain phenotypic differences in a population of cells. Because DNA, RNA, and proteins can be present and active at a few copies per cell, the abundance of gene products is theoretically sensitive to stochastic fluctuations. Four potential sources of variation in gene expression must be considered: (i) as described above, the inherent stochasticity of biochemical processes that are dependent on infrequent molecular events involving small numbers of molecules; (ii) variation in gene expression owing to differences in the internal states of a population of cells, either from predictable processes such as cell cycle progression or from a random process such as partitioning of mitochondria during cell division; (iii) subtle environmental differences, such as morphogen gradients in multicellular development; and (iv) ongoing genetic mutation, either random or directed. We use the term "noise" in gene expression to refer to the measured level of variation in gene expression among cells, regardless of source, within a supposedly identical population.

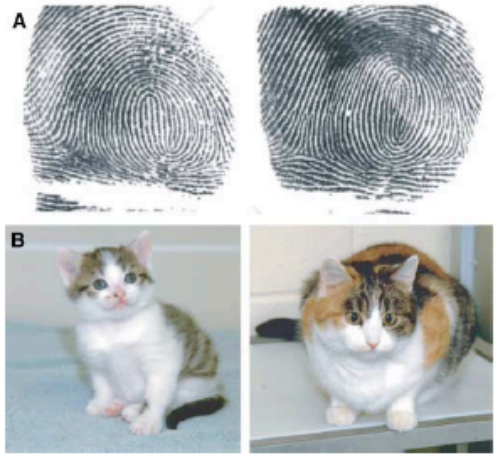

Fig. 1. Examples of possible stochastic influences on phenotype. (A) The fingerprints of identical twins are readily distinguished on close examination. Reprinted from (*37*) with permission from Elsevier. (B) Cc, the first cloned cat (left) and Rainbow, Cc's genetic mother (right), display different coat patterns and personalities (*38*). Photo credit, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University.

Measurement Techniques and Definitions

Recent investigations have employed green fluorescent protein (GFP) variants, which allow the quantification of protein levels in living cells by flow cytometry or fluorescence microscopy. The coefficient of variation, or noise η, is defined as the ratio of the standard deviation to the mean of the population. Other metrics of variability can be useful as well (SOM Text).

Once genetic mutation and local microenvironments are eliminated as sources of noise, an elegant experimental method can assist in differentiating among the remaining sources (*2*). This method involves quantifying expression of two equivalent, independent gene reporters placed in the same cell, which then allows noise sources to be partitioned into two categories: intrinsic, meaning noise sources that create differences between the two reporters within the same cell (Fig. 2A), and extrinsic, referring to sources that affect the two reporters equally in any given cell but create differences between two cells (Fig. 2B). Stochastic events during the process of gene expression, from the level of promoter-binding to mRNA translation to protein degradation, will manifest as intrinsic noise. Differences between cells, either in local environment or in the concentration or activity of any factor that affects gene expression, will result in extrinsic noise. Extrinsic noise should be further subdivided into two categories (*3*, *4*): global noise, or fluctuations in the rates of the basic reactions that affect expression of all genes (Fig. 2C), and gene- or pathway-specific extrinsic noise (Fig. 2D), such as fluctuations in the abundance of a particular transcription factor or stochastic events in a specific signal transduction pathway. If a factor that causes extrinsic noise is experimentally manipulable, it is possible to eliminate such extrinsic noise by reduction of variability in that factor; for example, cell cycle synchronization will reduce extrinsic

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Noise in genetic networks

Origins

How to measure and distinguish between the two types of noise (intrinsic vs extrinsic)?

What are the molecular processes that produce the most of noise?

Consequences

How is the noise propagated in gene networks?

How does the noise affect cellular behavior?

Control

What are the cellular mechanisms that confer robustness to noise?

Origin of noise in genetic networks

Stochasticity gene expression in a single cell
Elowitz, Levine, Siggia, Swain (2002) ***Science*** **297: 1183-86**

Regulation of noise in the expression of a single gene
Ozdudak, Thattai, Kurtser, Grossman, van Oudenaarden (2002) ***Nat Genet*** **31: 69-73**

Control of stochasticity in eukaryotic gene expression
Raser, O'Shea (2004) ***Science*** **304: 1811-14**

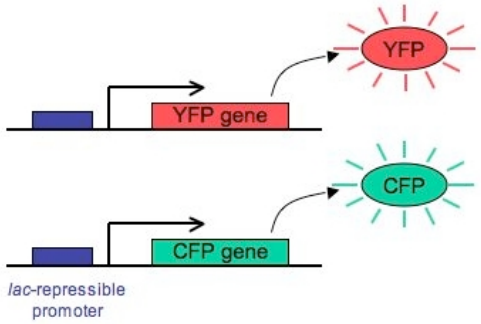
Noise in eukaryotic gene expression
Blake, Kaern, Cantor, Collins (2003) ***Nature*** **422: 633-637**

Gene regulation at the single-cell level
Rosenfeld, Young, Alon, Swain, Elowitz (2005) ***Science*** **307: 1962-1965**

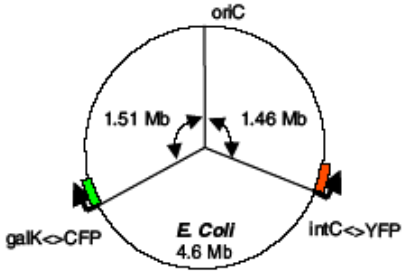
Stochasticity gene expression in a single cell

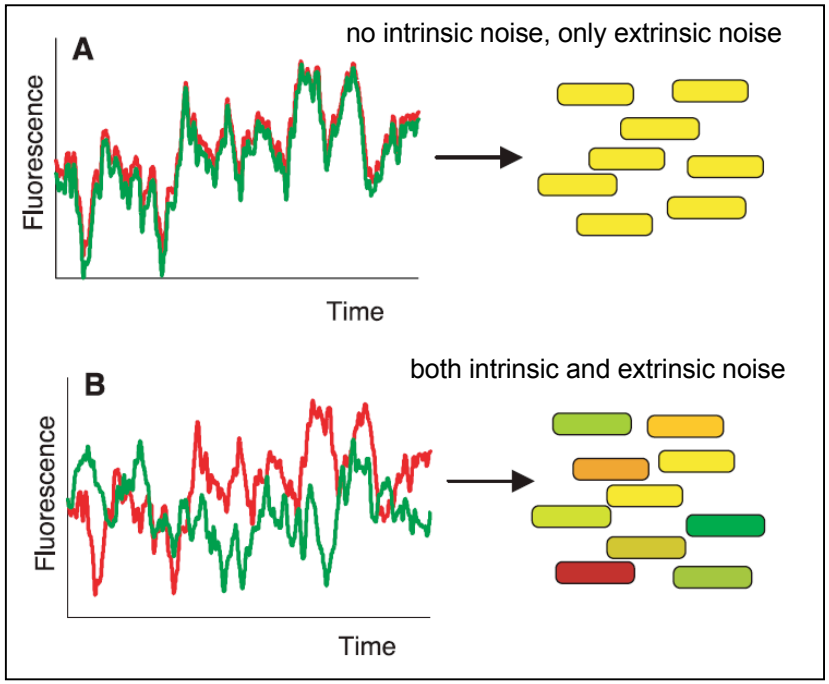
Elowitz, Levine, Siggia, Swain (2002) *Science* 297: 1183-86

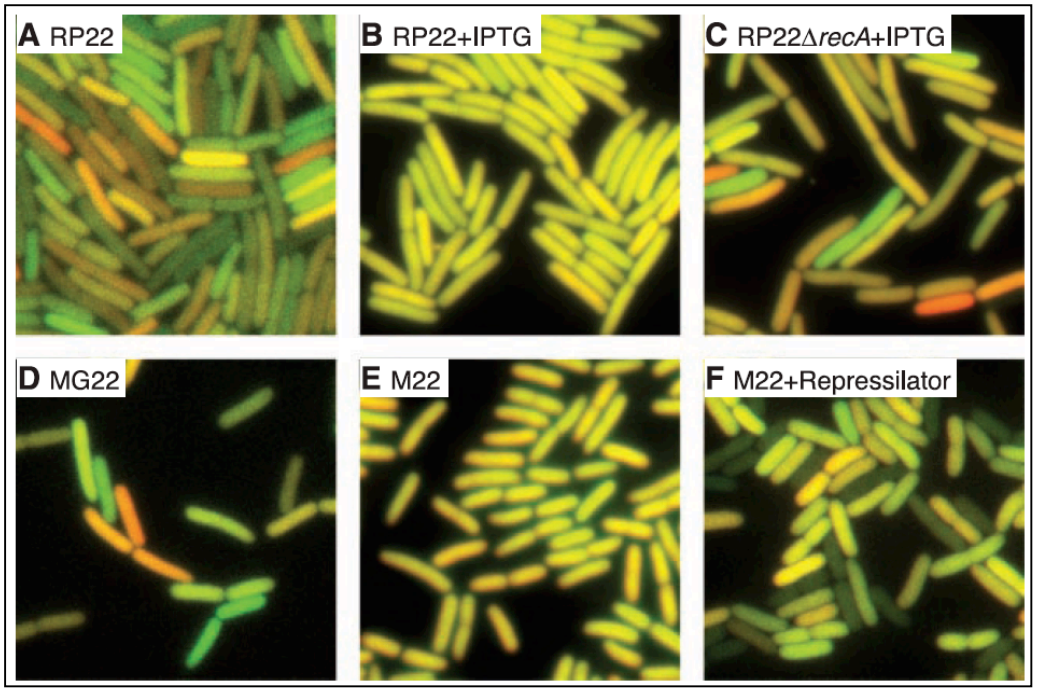
Double reporter construction



Experiment in *E. coli*







Stochasticity gene expression in a single cell

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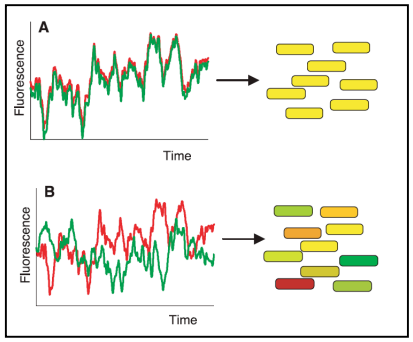
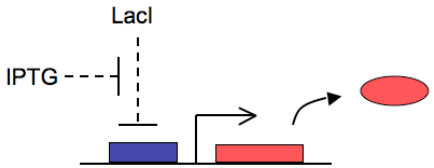
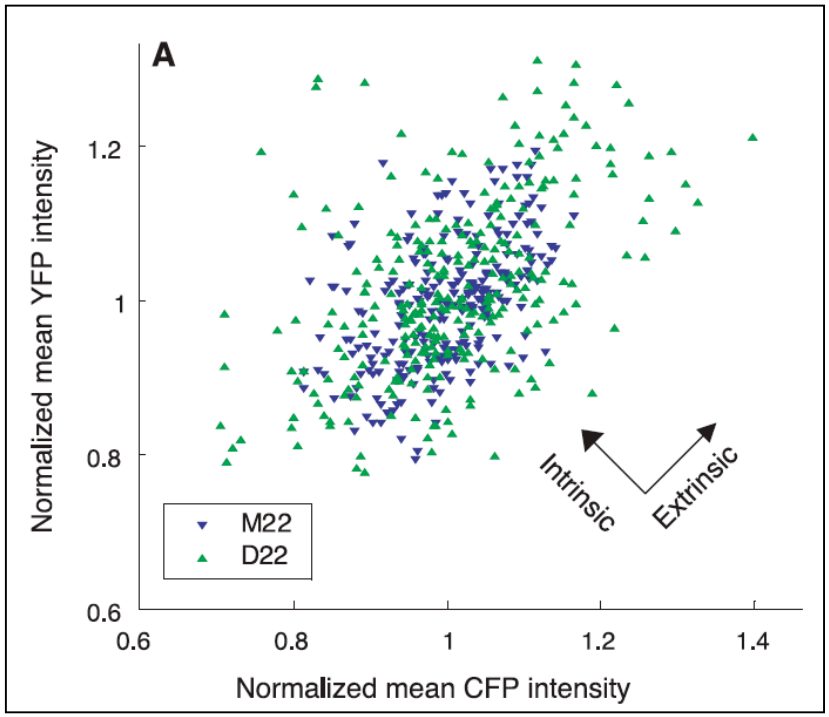
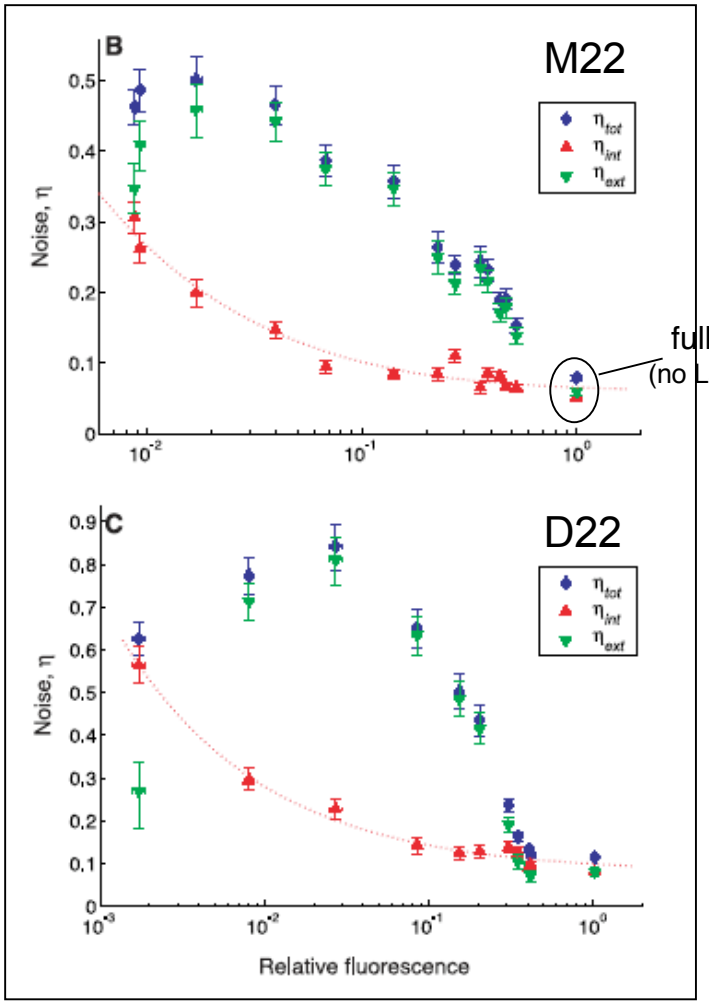


Conrol of gene expression







fully induced
(no LacI repressor)

Expression level controled by IPTG

M22 = quite strain (wild type)
D22 = noisy strain (deletion of recA gene)

Stochasticity gene expression in a single cell

Elowitz, Levine, Siggia, Swain (2002) *Science* 297: 1183-86

Conclusions

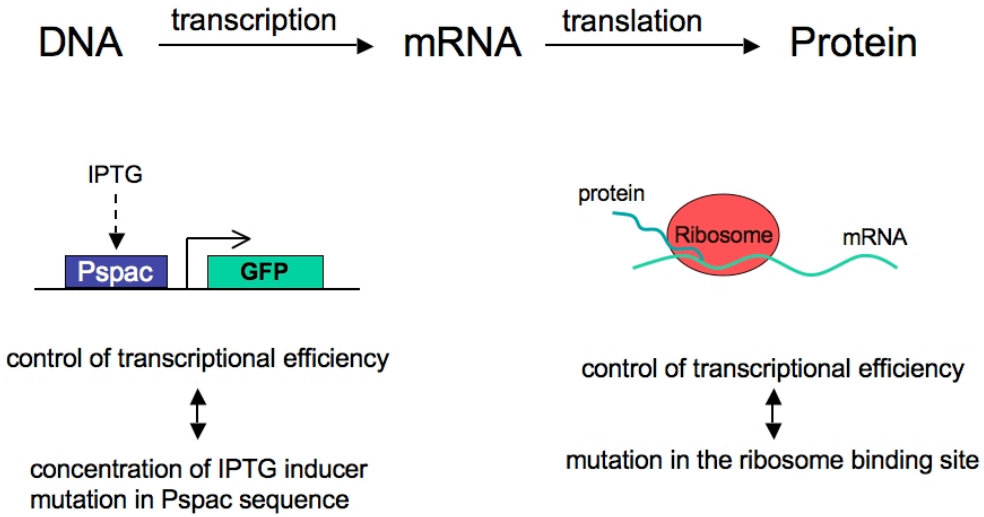
- Using a two-reporter method, it is possible to measure and distinguished between extrinsic and intrinsic noise.
- The stochastic nature of gene expression gives rise to noise in protein levels.
- The relative contributions of extrinsic and intrinsic component to the total noise vary with expression level.
- An increase of noise may arise from transient copy number differences between parts of the chromosomes

For the theory see: Swain, Elowitz, Siggia (2002) Intrinsic and extrinsic contributions to stochasicity in gene expression. *PNAS* 99: 12795-801

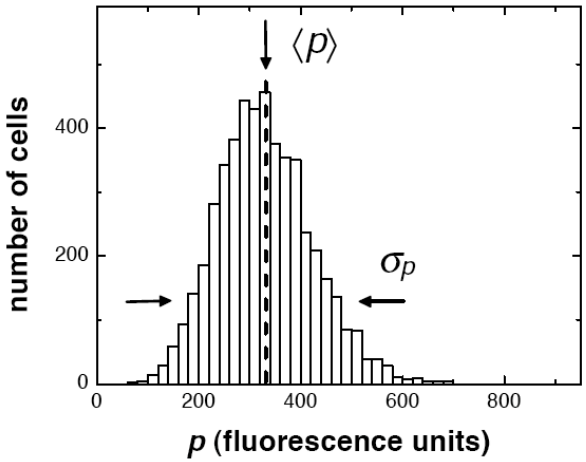
Regulation of noise in the expression of a single gene

Ozbudak, Thattai, Kurtser, Grossman, van Oudenaarden (2002) *Nat Genet* 31: 69-73

Methodology



Experiment in *bacilius subtilis*



Translational and transcriptional mutants

Table 1 • Translational mutants: point mutations in the RBS and initiation codon of *gfp*

Strain	Ribosome binding site	Initiation codon	Translational efficiency
ERT25	GGG AAA AGG AGG TGA ACT ACT	ATG	1.00
ERT27	GGG AAA AGG AGG TGA ACT ACT	TTG	0.87
ERT3	GGG AAA AGG TGG TGA ACT ACT	ATG	0.84
ERT29	GGG AAA AGG AGG TGA ACT ACT	GTG	0.66

Table 2 • Transcriptional mutants: point mutations in the P_{spac} promoter

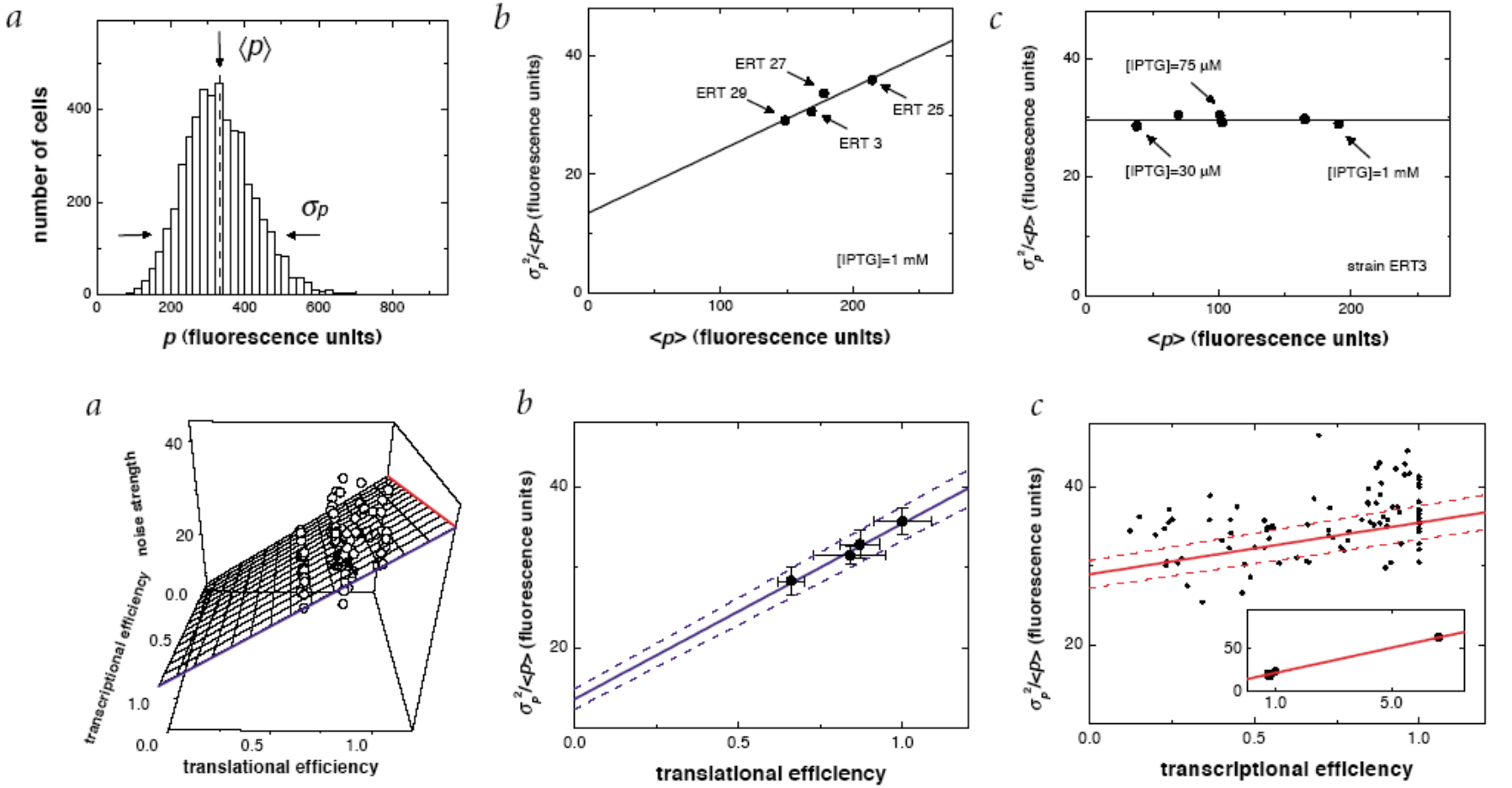
Strain	−10 regulatory region −10 +1	Transcriptional efficiency
ERT57	CAT AAT GTG TGT AAT	6.63
ERT25	CAT AAT GTG TGG AAT	1.00
ERT53	CAT AAT GTG TGC AAT	0.79
ERT51	CAT AAT GTG TGA AAT	0.76
ERT55	CAT AAT GTG TAA AAT	0.76

Regulation of noise in the expression of a single gene

Ozbudak, Thattai, Kurtser, Grossman, van Oudenaarden (2002) *Nat Genet* 31: 69-73

Various translational mutants

Various transcriptional mutants



Regulation of noise in the expression of a single gene

Ozbudak, Thattai, Kurtser, Grossman, van Oudenaarden (2002) *Nat Genet* 31: 69-73

Theoretical model

mRNA

$$\frac{dr}{dt} + \gamma_R r = k_R + \eta_R$$

protein

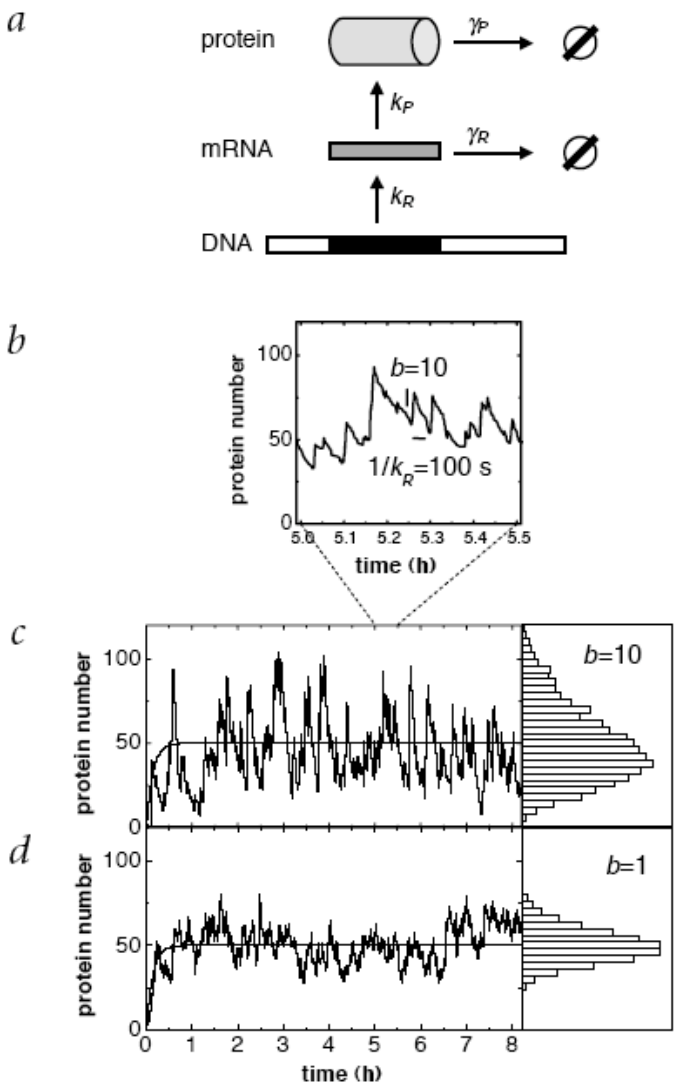
$$\frac{dp}{dt} + \gamma_P p = k_P r + \eta_P$$

average number of proteins synthesized per mRNA transcript

$$b = k_P / \gamma_R$$

mean level of protein and standard deviation (noise)

$$\langle p \rangle = k_R b / \gamma_P \qquad \frac{\sigma_p^2}{\langle p \rangle} \cong 1 + b$$



Regulation of noise in the expression of a single gene

Ozbudak, Thattai, Kurtser, Grossman, van Oudenaarden (2002) *Nat Genet* 31: 69-73

Conclusions

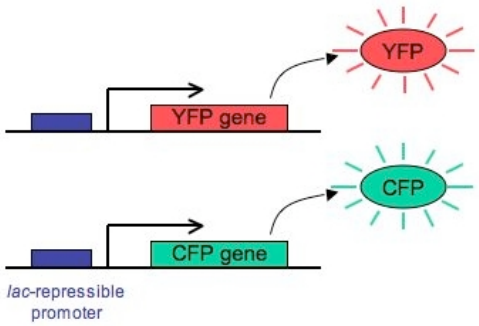
- Translation is the dominant source of noise in protein levels.
- This result is consistent with the prediction of a simple theoretical model of stochastic gene expression.

For the theory see: Thattai, van Oudenaarden (2002) Intrinsic noise in gene regulatory networks. *PNAS* 98: 8614-8610

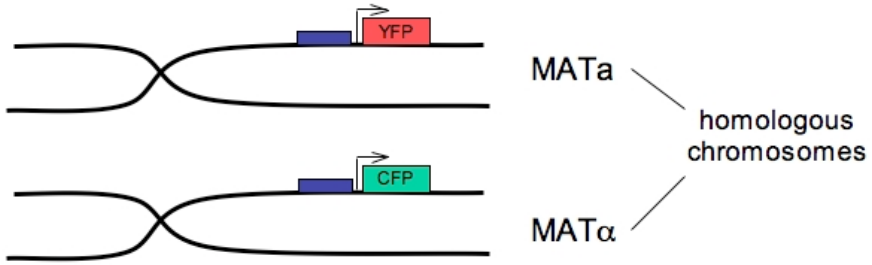
Control of stochasticity in eukaryotic gene expression

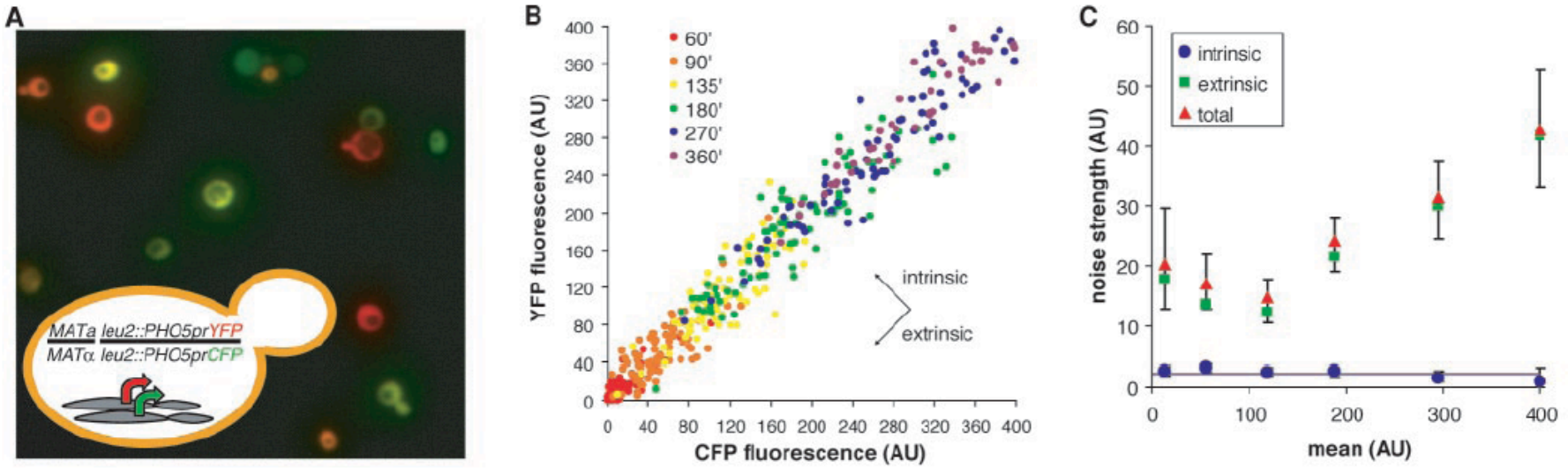
Raser, O'Shea (2004) *Science* 304: 1811-14

Double reporter construction



Experiment in *S. cerevisiae*





Control of stochasticity in eukaryotic gene expression

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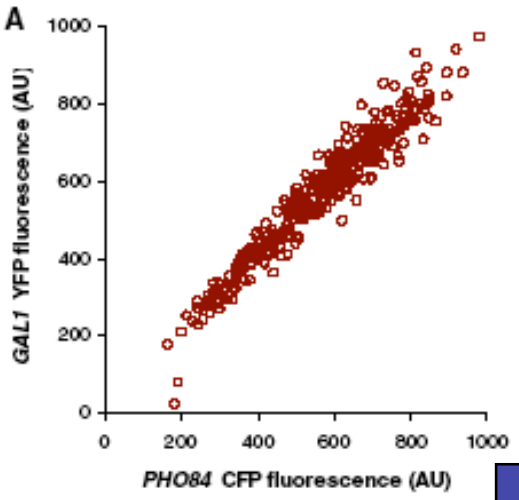
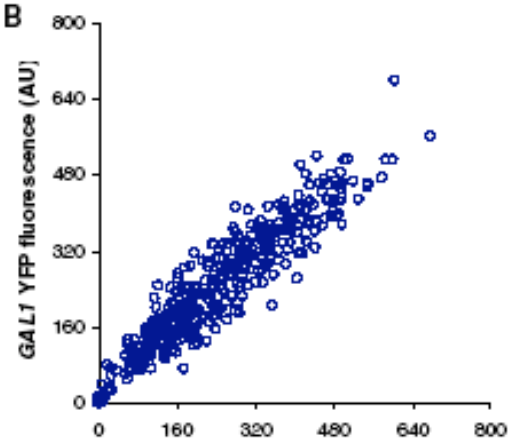
ADH1



PHO84

GAL1



PHO84

Experiment with different promoters:

total (≈ extrinsic) noise

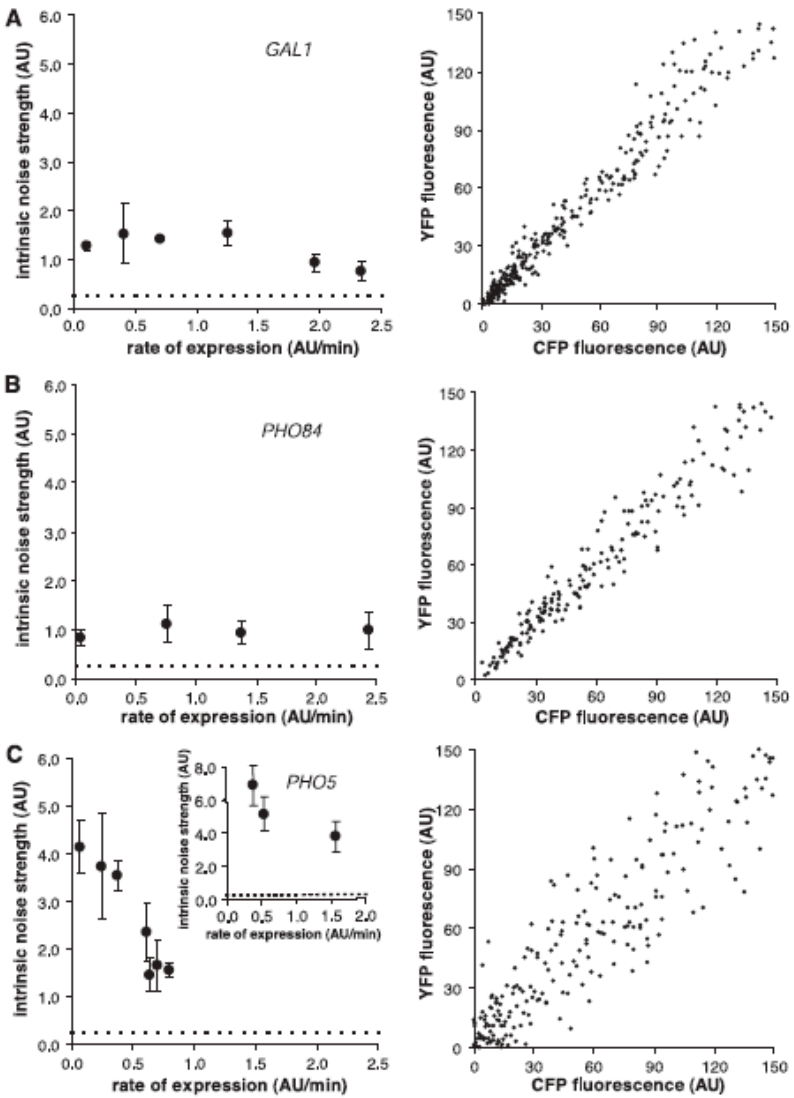
Control of stochasticity in eukaryotic gene expression

Raser, O'Shea (2004) *Science* 304: 1811-14

GAL1

PHO84

PHO5



Experiment with different promoters: intrinsic noise

GAL1 promoter — controled by Gal4p

PHO84 promoter, PHO5 promoter — controled by the same activator (Pho4p)

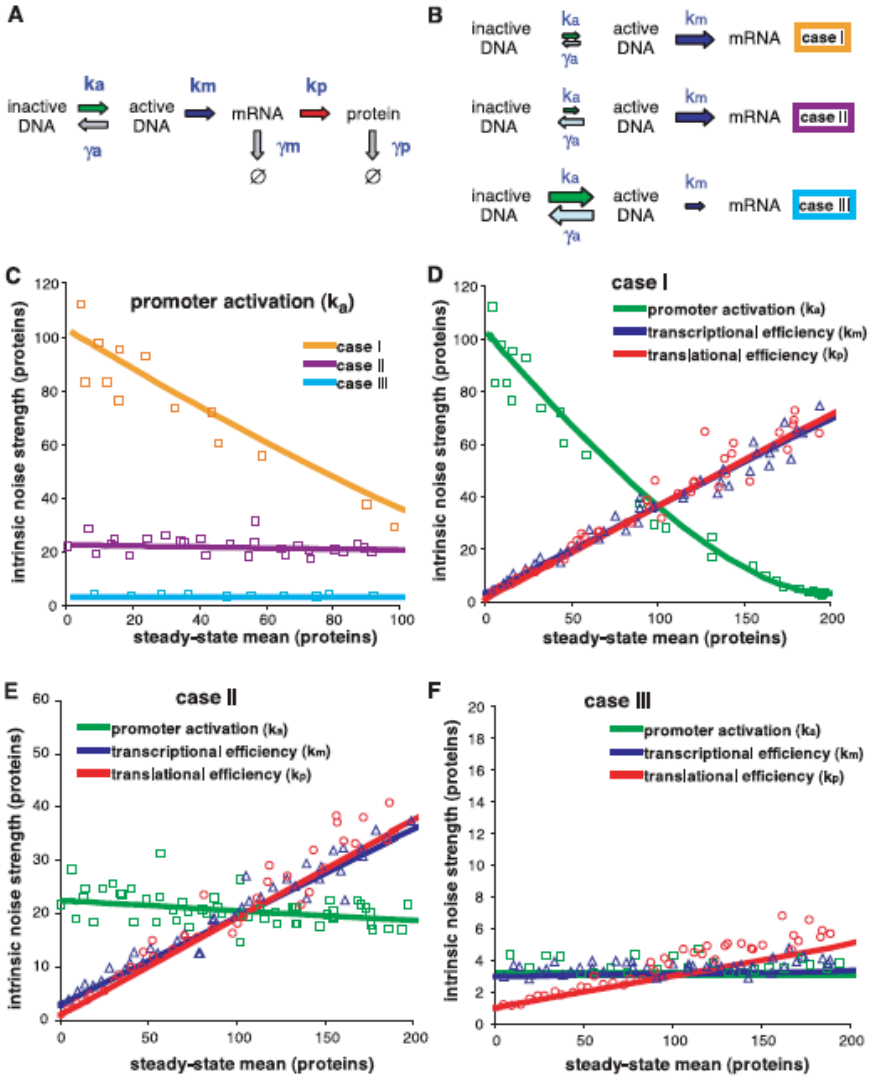
Observation

GAL1 promoter, PHO84 promoter — low intrinsic noise

PHO5 promoter — larger intrinsic noise

Control of stochasticity in eukaryotic gene expression

Raser, O'Shea (2004) *Science* 304: 1811-14



Theoretical model

Comparison of various models of gene activation

The noise strength profile of PHO5 is similar to the prediction made for case I (see panel D) when the promoter activation rate is changed (see green curve).

Prediction:

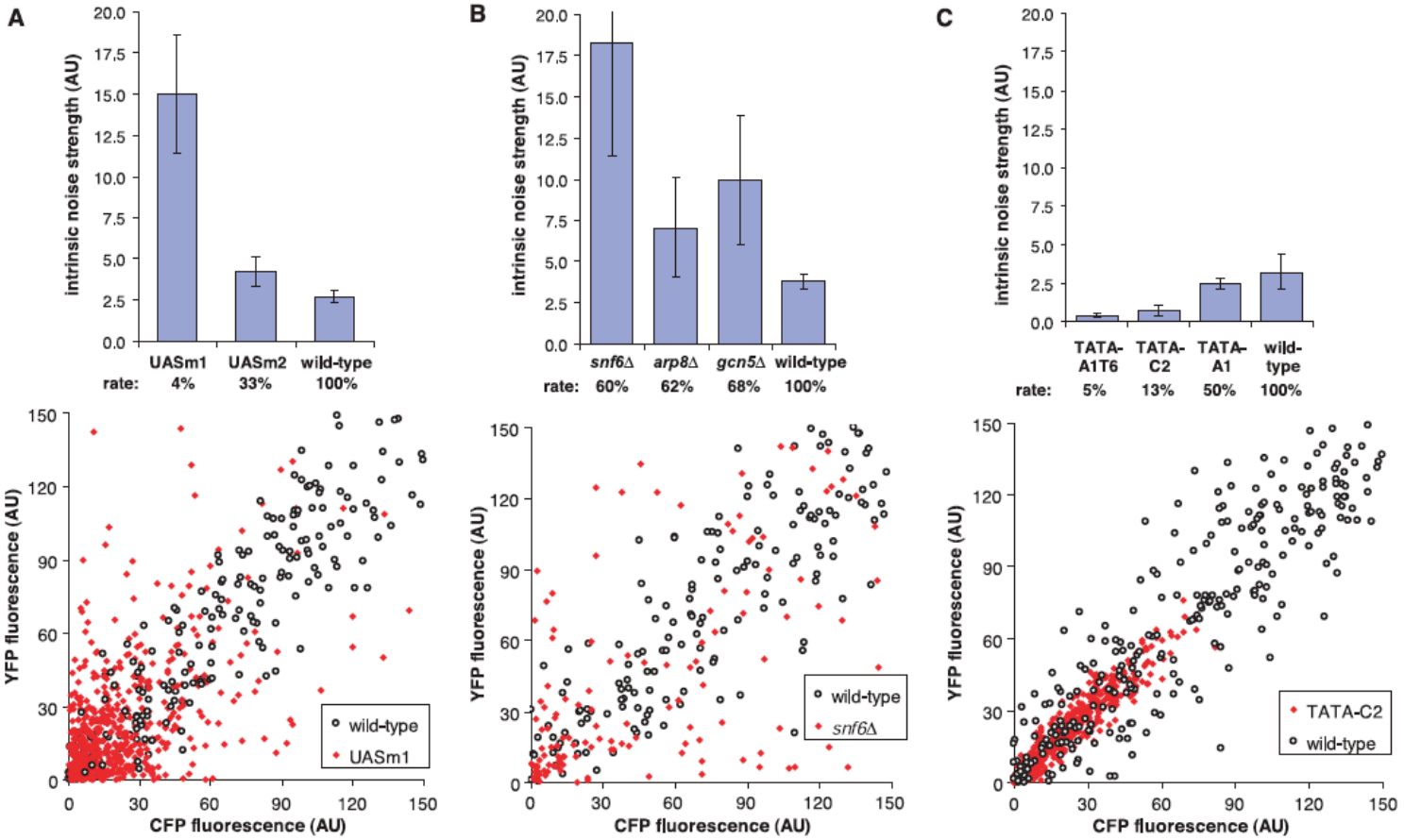
The noise generation in PHO5 is dependent on the rate of a slow upstream promoter transition

Control of stochasticity in eukaryotic gene expression

Raser, O'Shea (2004) *Science* 304: 1811-14

complexes involved in chromatin remodeling — **transcription efficiency**

mutations in UAS — **mutations in snf6, arp8,...** — **mutations in TATA box**



Control of stochasticity in eukaryotic gene expression

Raser, O'Shea (2004) *Science* 304: 1811-14

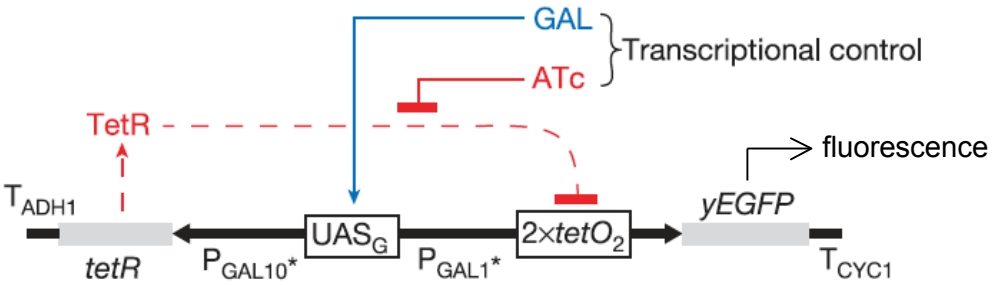
Conclusions

- The two-reporter technique can be applied to eukaryotes (yeast).
- Extrinsic noise is predominant over intrinsic noise
- Total noise (≈ extrinsic) is not gene-specific, but intrinsic noise is gene-specific.
- Noise does not depend on the regulatory pathway, neither on absolute rate of expression.
- Noise depends on the rate of a slow upstream promoter transition, such as chromatine remodeling

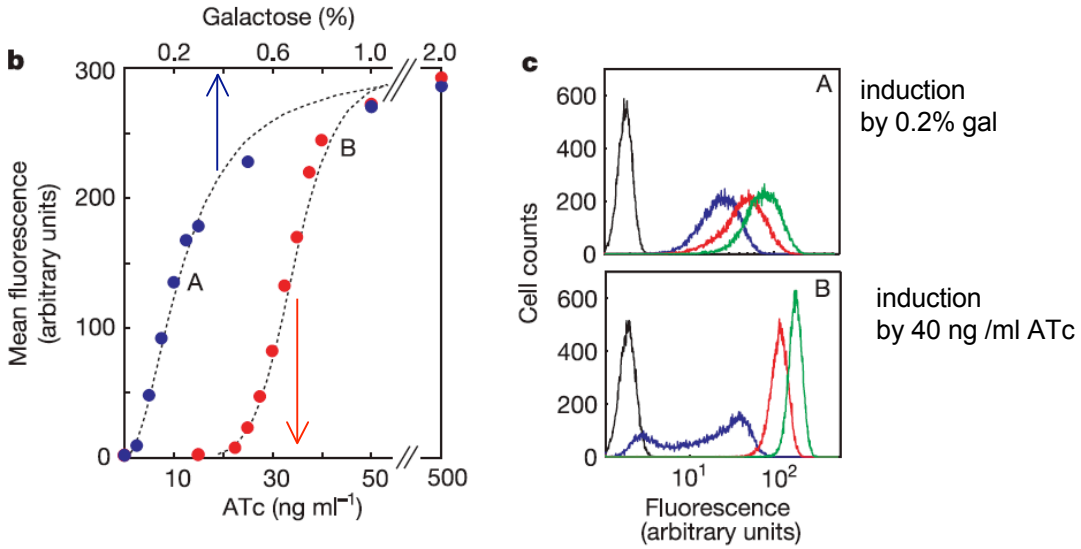
Noise in eukaryotic gene expression

Blake, Kaern, Cantor, Collins (2003) *Nature* 422: 633-637

Experiment in *S. cerevisiae*



Genetic construction with two transcriptional controls:
GAL (direct activator)
and
ATc (inhibitor of the TetR inhibitor)



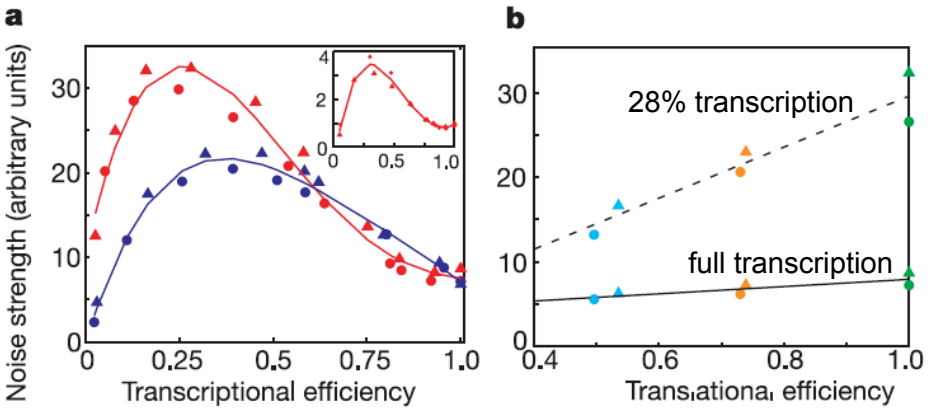
Inductions by GAL or ATc induce differential responses.

The mode of transcriptional control has thus a significant influence on the response to the noise.

Noise in eukaryotic gene expression

Blake, Kaern, Cantor, Collins (2003) *Nature* 422: 633-637

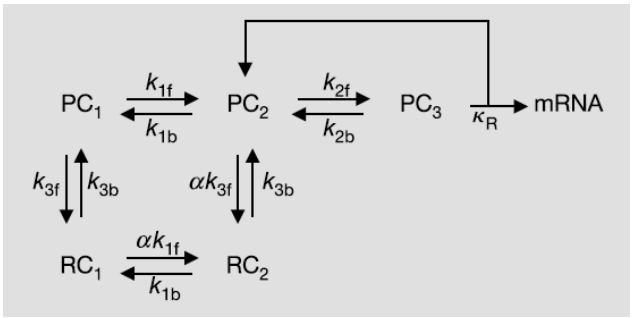
Experiment: effect of transcriptional and translational efficiency on the noise

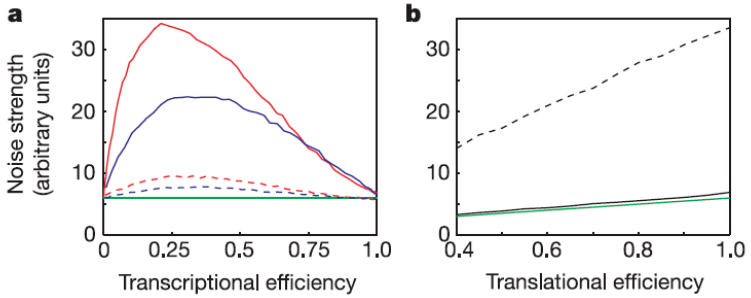


Controled by
ATc or GAL

Controled by
mutation in codons
(by keeping the same
aa sequence)

Model

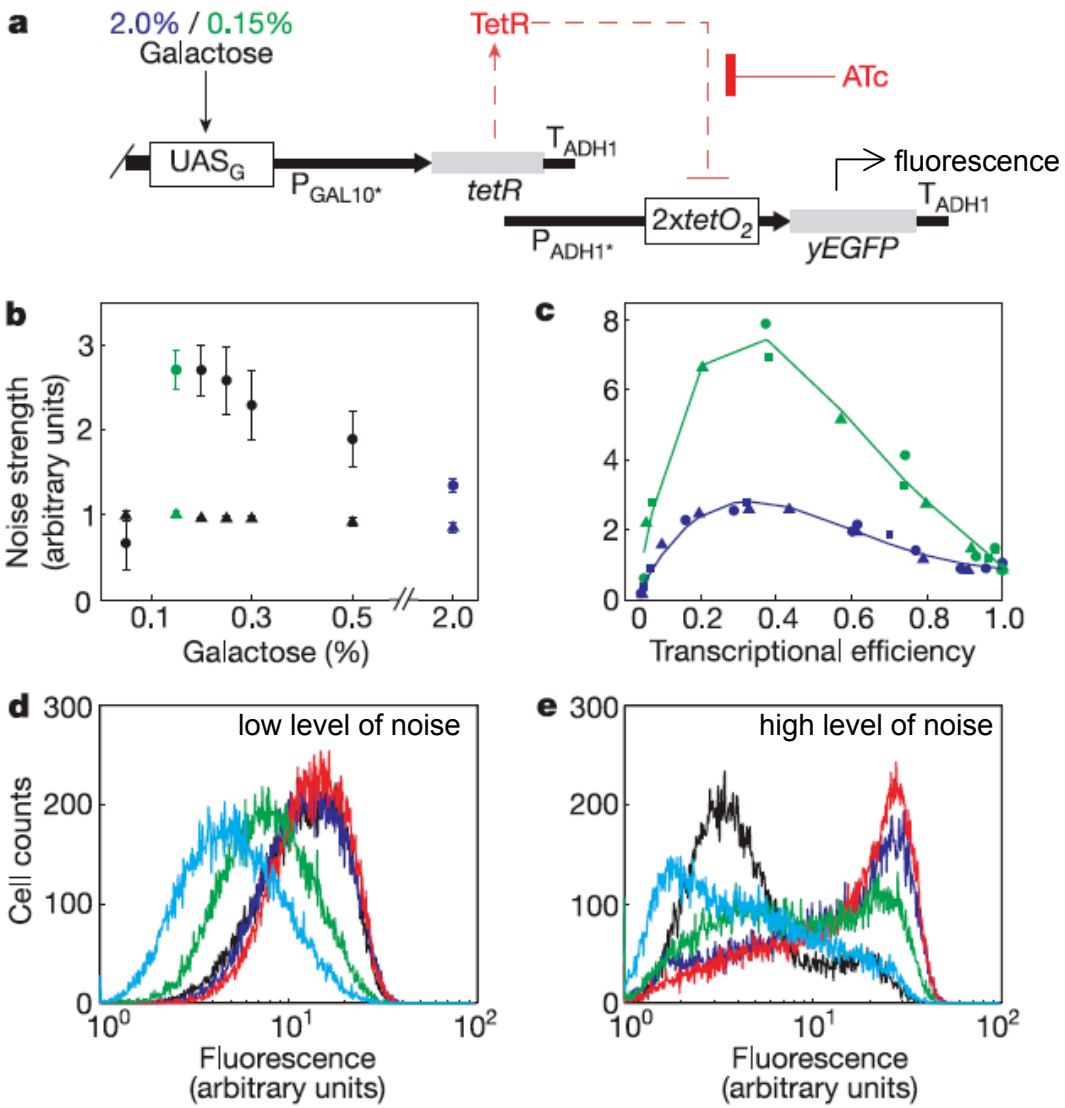




The level of noise in eukaryotic gene expression is strongly influenced by transcription

Noise in eukaryotic gene expression

Blake, Kaern, Cantor, Collins (2003) *Nature* 422: 633-637



Experiment: cascading noise in a gene network

Downstream effects of noise can have profound phenotypic consequences, drastically affecting the stability of gene expression

Noise in eukaryotic gene expression

Blake, Kaern, Cantor, Collins (2003) *Nature* 422: 633-637

Conclusions

- In eukaryots, the mode of transcriptional control can have a marked effect on the response to the noise.
- In eukaryots, noise arising from transcription contributes more than noise generated at the translational level (in contrast to observation in prokaryots).
- Downstream effects of noise can have profound phenotypic consequences, drastically affecting the stability of gene expression.

Consequence of noise in genetic networks

Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *E. coli* cell
Arkin, Ross, McAdams (1998) *Genetics* 149: 1633-48

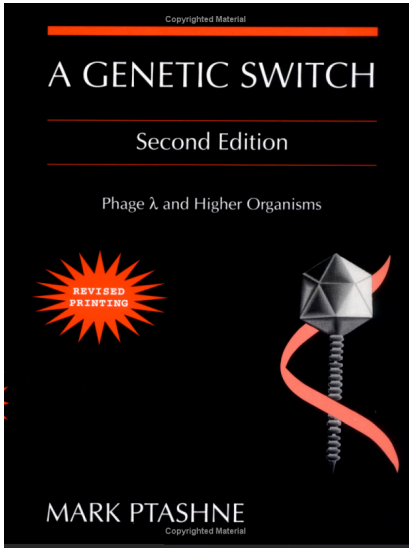
Multistability in the lactose utilization network of *E. coli*
Ozbudak, Thaittai, Lim, Shraiman, van Oudenaarden (2004) *Nature* 427: 737-740

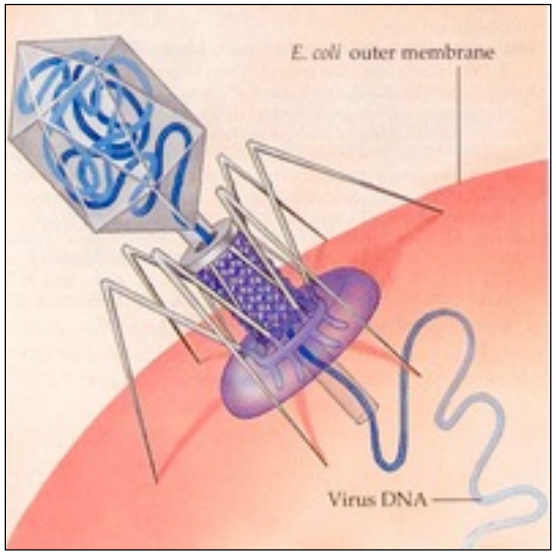
Noise propagation in gene networks
Pedraza, van Oudenaarden (2005) *Science* 307: 1965-69

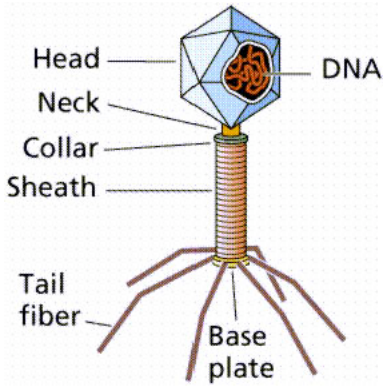
Ultrasensitivity and noise propagation in a synthetic transcriptional cascade
Hooshangi, Thilberge, Weiss (2005) *PNAS* 102: 3581-3586

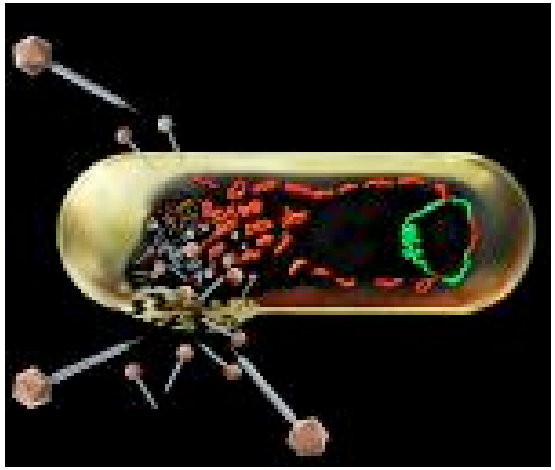
Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *Escherichia coli* cell

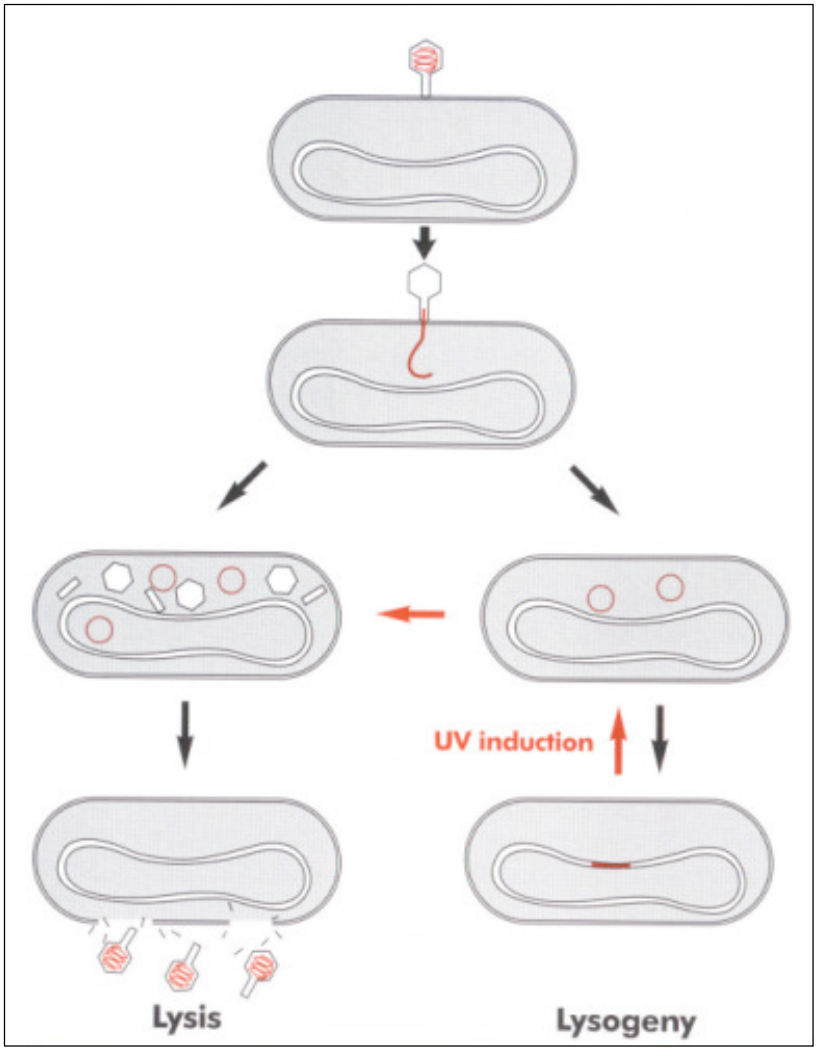
Arkin, Ross, McAdams (1998) *Genetics* 149: 1633-48











Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *Escherichia coli* cell

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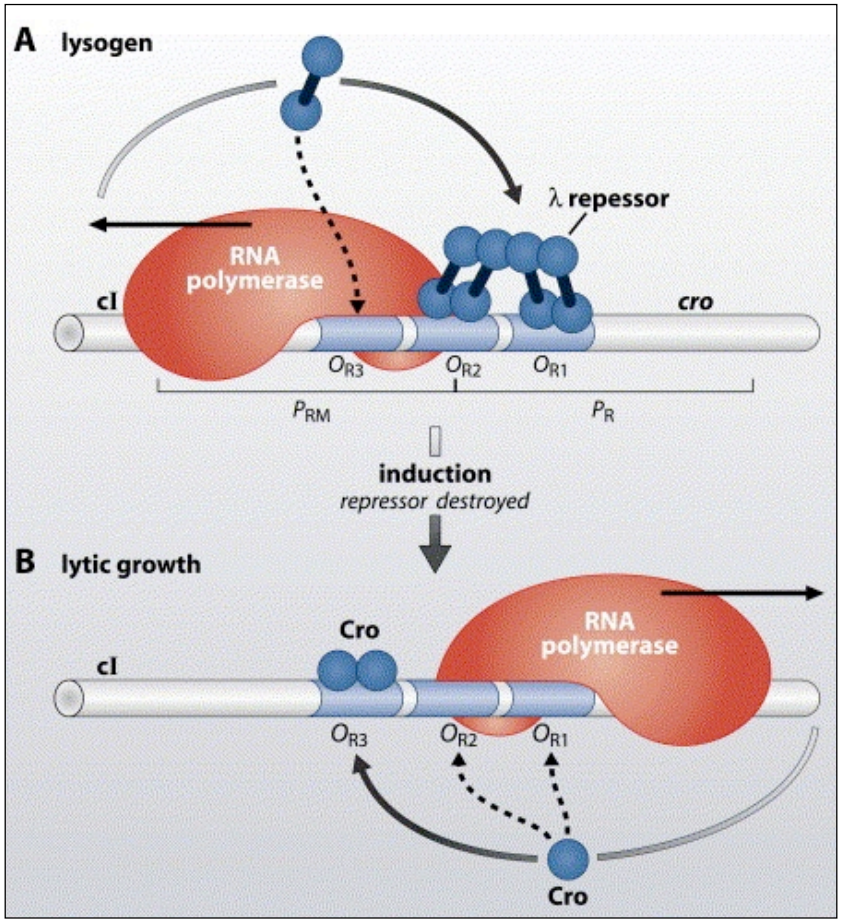
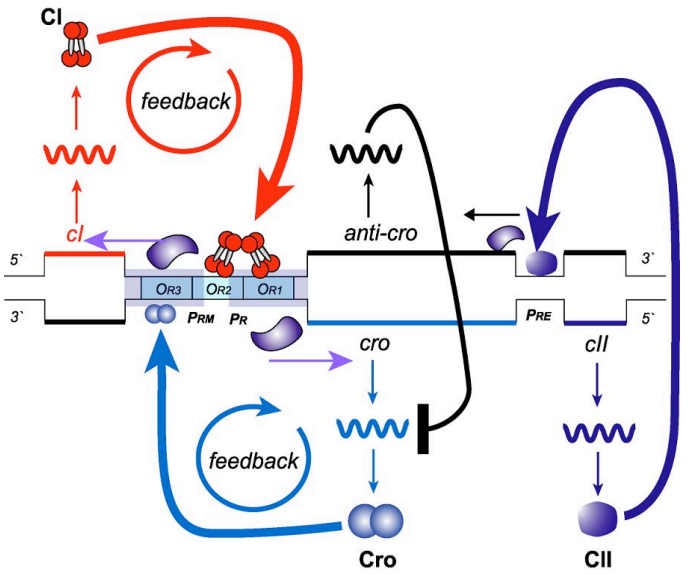


Switch CRO / CII

high CRO, low CI → lysis
low CRO, high CI → lysogeny



Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *Escherichia coli* cell

Arkin, Ross, McAdams (1998) *Genetics* 149: 1633-48

Very detailed molecular model for the phage-λ genetic switch

Parameters for transcription and translation reactions

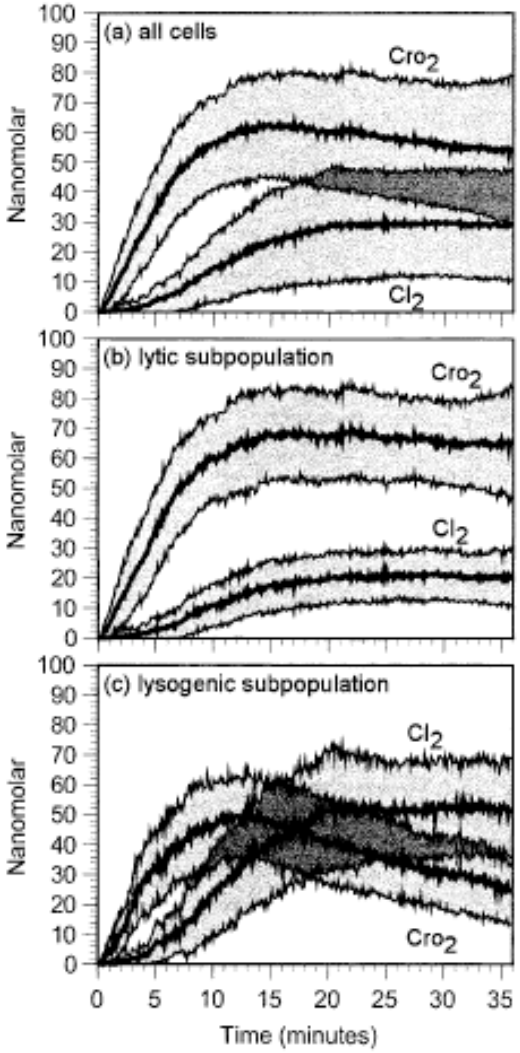
Reaction/event	Parameter	References and comments
Transcription reactions		
$RNAP{\cdot}DNA_n \xrightarrow{k_{22}} RNAP{\cdot}DNA_{n+1}$	$k_{22} = 30$ nt sec^{-1}	Selected as an average rate. Measured elongation rates vary widely, depending on DNA template and cell state (Gotta *et al.* 1991; Kennell and Riezman 1977; Kornberg and Baker 1992; Vogel and Jensen 1994)
$RNAP{\cdot}DNA_{Nut(L,R)} \xrightarrow{k_{23}} RNAP{\cdot}DNA_{Nut(L,R)+1}$	$k_{23} = 5$ nt sec^{-1}	
$RNAP{\cdot}DNA_{Nut(L,R)} + N \underset{k_{25}}{\overset{k_{24}}{\rightleftharpoons}} RNAP{\cdot}N{\cdot}DNA_{Nut(L,R)+1}$	$k_{24} = 0.145$ (nM sec)$^{-1}$ $k_{25} = 0.1$ sec^{-1}	Selected to produce termination and antitermination consistent with Li *et al.* (1992) and Whalen *et al.* (1988)
$RNAP{\cdot}N{\cdot}DNA_{Nut(L,R)} \xrightarrow{k_{26}} RNAP{\cdot}N{\cdot}DNA_{Nut(L,R)+1}$	$k_{26} = 30$ nt sec^{-1}	
$RNAP{\cdot}DNA_{T1t} \xrightarrow{k_{27}} RNAP{\cdot}DNA_{T1t+1}$	$k_{27} = 15$ nt sec^{-1}	Selected to yield 50% termination at $N = 0$ nM (Dambly-Chaudiere *et al.* 1983; Friedman and Gottesman 1983)
$RNAP{\cdot}DNA_{T1t} \xrightarrow{k_{28}} RNAP + DNA_{T1t}$	$k_{28} = 15$ sec^{-1}	
$RNAP{\cdot}N{\cdot}DNA_{T1t} \xrightarrow{k_{29}} RNAP{\cdot}N{\cdot}DNA_{T1t+1}$	$k_{29} = 30$ nt sec^{-1}	Assumption that antiterminated RNAP passes terminator freely
$RNAP{\cdot}DNA_{T1i} \xrightarrow{k_{31}} RNAP{\cdot}DNA_{T1i+1}$	$k_{31} = 5$ nt sec^{-1}	Selected to yield 80% termination at $N = 0$ nM
$RNAP{\cdot}DNA_{T1i} \xrightarrow{k_{32}} RNAP + DNA_{T1i}$	$k_{32} = 25$ sec^{-1}	Selected to yield 80% termination at $N = 0$ nM
$RNAP{\cdot}N{\cdot}DNA_{T1i} \xrightarrow{k_{33}} RNAP{\cdot}N{\cdot}DNA_{T1i+1}$	$k_{33} = 30$ nt sec^{-1}	Assumption: antiterminated RNAP passes terminator freely
Translation reactions		
$Ribosome + RNA_{RBS} \xrightarrow{k_{34}} Ribosome{\cdot}RNA_{RBS}$	$k_{34} = 0.002$ (nM sec)$^{-1}$	(Kennell and Riezman 1977; Sorensen and Pedersen 1991)
$Ribosome + RNA_n \xrightarrow{k_{35}} Ribosome{\cdot}RNA_{n+1}$	$k_{35} = 100$ nt sec^{-1}	(Adhya and Gottesman 1982; Kennell and Riezman 1977; Sorensen and Pedersen 1991)
$RNase + RNA_{RBS} \xrightarrow{k_{36}} RNase$	k_{36}·RNase $= 0.2$ sec^{-1}	Adjusted to get an average of 10 proteins per transcript
Average number of proteins per transcript (all transcripts)	10	(Kepes 1963; Yarchuk *et al.* 1992)

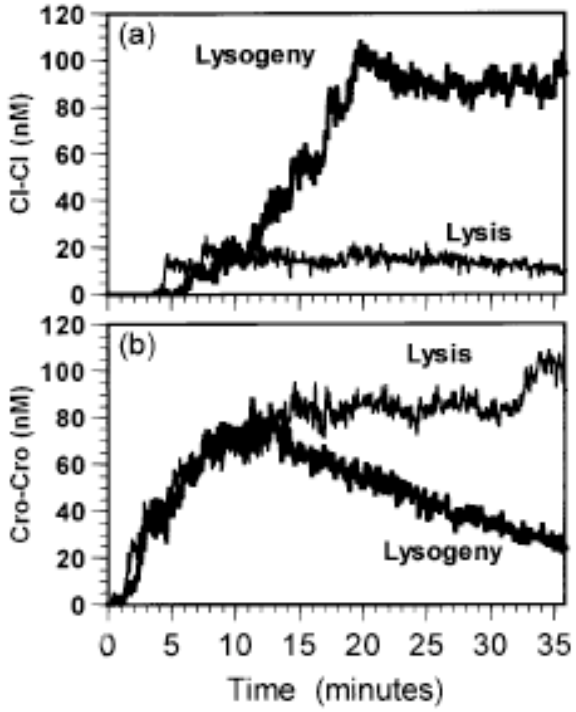
Parameters for housekeeping and nongenetic reactions

Reaction/event	Parameter	References and comments
Housekeeping reactions		
Available RNAP	RNAP = 30 nM	McClure (1980, 1983)
Available ribosomes	Ribosomes = 500 nM	
Cell volume $(t) = (1 + k_9 * t) \times 10^{-15}$ liters	$k_9 = 4.76 \times 10^{-18}$ liters sec^{-1}	To double initial cell volume of 10^{-15} liters in 35 min
Nongenetic reactions[a]		
$CI \xrightarrow{k_1} ( )$	$k_1 = 0.0007$ sec^{-1}	Selected to yield a CI/CI_2 life time of approximately 40 min (Reinitz and Vaisnys 1990) in the concentration range between 20 and 100 nM
$2{\cdot}CI \underset{k_3}{\overset{k_2}{\rightleftharpoons}} CI_2$	$k_2 = 0.05$ M^{-1} sec^{-1}	Burz *et al.* (1994); Shea and Ackers (1985)
	$k_3 = 0.5$ sec^{-1}	
$Cro \xrightarrow{k_4} ( )$	$k_4 = 0.0025$ sec^{-1}	Selected to match Cro/Cro_2 lifetime of approximately 30 min (Reinitz and Vaisnys 1990) in the concentration range between 20 and 100 nM
$2{\cdot}Cro \underset{k_6}{\overset{k_5}{\rightleftharpoons}} Cro_2$	$k_5 = 0.05$ M^{-1}sec^{-1}	Reinitz and Vaisnys (1990); Sauer (1979)
	$k_6 = 0.5$ sec^{-1}	
$N \xrightarrow{k_7} ( )$	$k_7 = 0.00231$ sec^{-1}	Gottesman and Gottesman (1981)
P1 concentration[b]	*P1* = 35 nM	Adjusted to match the % lysogeny vs. API data (Kourilsky 1973)
$CII + P1 \underset{k_9}{\overset{k_8}{\rightleftharpoons}} P1{\cdot}CII$	$k_8 = 0.01$ M^{-1} sec^{-1}	Selected to match CII half-life in Gottesman and Gottesman (1981)
$P1{\cdot}CII \xrightarrow{k_{10}} P1$	$k_9 = 0.01$ sec^{-1}	
	$k_{10} = 0.002$ sec^{-1}	
$CIII + P1 \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} P1{\cdot}CIII$	$k_{11} = 0.01$ M^{-1} sec^{-1}	Selected to match CIII protection of CII degradation (Hoyt *et al.* 1982; Rattray *et al.* 1984) and CIII half-life Kornitzer *et al.* (1991a,b)
$P1{\cdot}CIII \xrightarrow{k_{13}} P1$	$k_{12} = 0.001$ sec^{-1}	
	$k_{13} = 0.0001$ sec^{-1}	
P2 concentration	*P2* = 140 nM	
$CII + P2 \underset{k_{15}}{\overset{k_{14}}{\rightleftharpoons}} P2{\cdot}CII$	$k_{14} = 0.00025$ M^{-1}sec^{-1}	Selected to match CII half-life in Gottesman and Gottesman (1981)
$P2{\cdot}CII \xrightarrow{k_{16}} P2$	$k_{15} = 0.065$ sec^{-1}	
	$k_{16} = 0.6$ sec^{-1}	
$CIII + P2 \underset{k_{18}}{\overset{k_{17}}{\rightleftharpoons}} P2{\cdot}CIII$	$k_{17} = 0.01$ M^{-1} sec^{-1}	Selected to match CIII protection of CII from degradation (Hoyt *et al.* 1982; Rattray *et al.* 1984) and CIII half-life (Kornitzer *et al.* 1991a,b)
$P2{\cdot}CIII \xrightarrow{k_{19}} P2$	$k_{18} = 0.01$ sec^{-1}	
	$k_{19} = 0.001$ sec^{-1}	

Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *Escherichia coli* cell

Arkin, Ross, McAdams (1998) *Genetics* 149: 1633-48





The **random developmental path choice** between the lysogenic or lytic path in individual cells results from the inevitable **fluctuations** in the temporal pattern of **protein concentration** growth caused by the molecular-level thermal fluctuations in rates of rate-determining reactions within gene expression mechanisms.

The resulting differences in concentration between the regulatory proteins controlling the bistable switching elements of the decision circuit led to different path selections in different cells.

Stochastic kinetic analysis of a developmental pathway bifurcation in phage-λ *Escherichia coli* cell

Arkin, Ross, McAdams (1998) *Genetics* 149: 1633-48

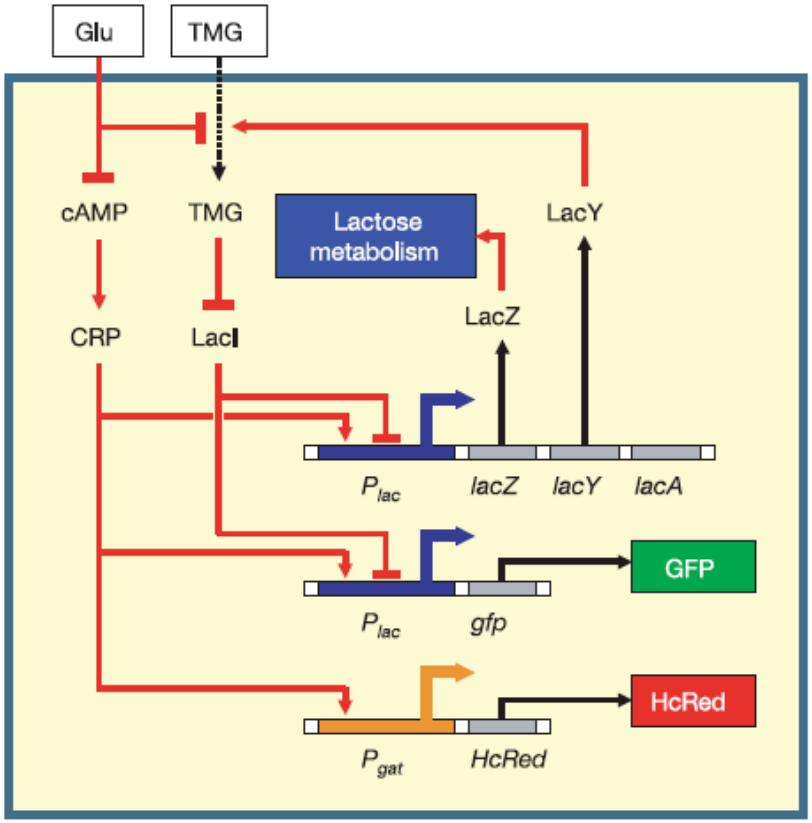
Conclusions

- Stochastic variations at the genetic level can produce probabilistic pathway selection, thereby leading to distinct phenotypic subpopulations.

Multistability in the lactose utilization network of *E. coli*

Ozbudak, Thaittai, Lim, Shraiman, van Oudenaarden (2004) *Nature* 427: 737-740

Lactose utilization network in *E. coli*



TMG = inducer
= control parameter
(inhibits LacI inhibitor)

GFP = green fluorescent reporter,
controled by P_{lac} promoter

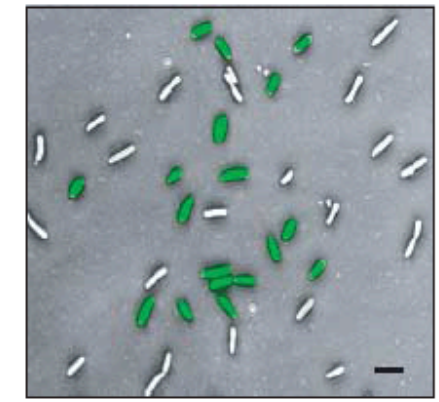
Multistability in the lactose utilization network of *E. coli*

Ozbudak, Thaittai, Lim, Shraiman, van Oudenaarden (2004) *Nature* 427: 737-740

Bistability and hysteresis effect in single cells

Some cell are fully induced (green) or fully uninduced (white)

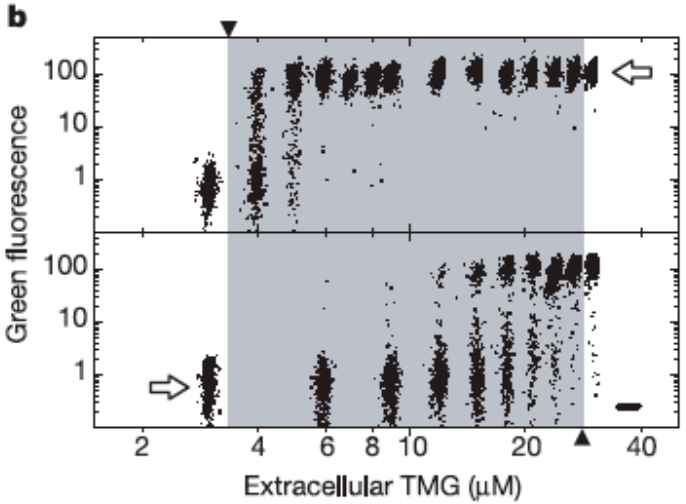
(bistability)

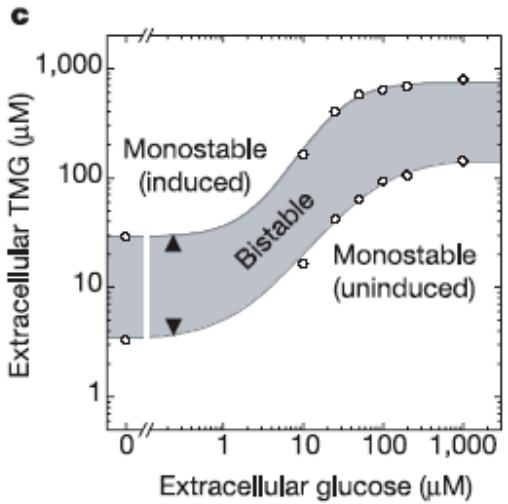


18 μM TMG

Induction by TMG

starting from induced cells

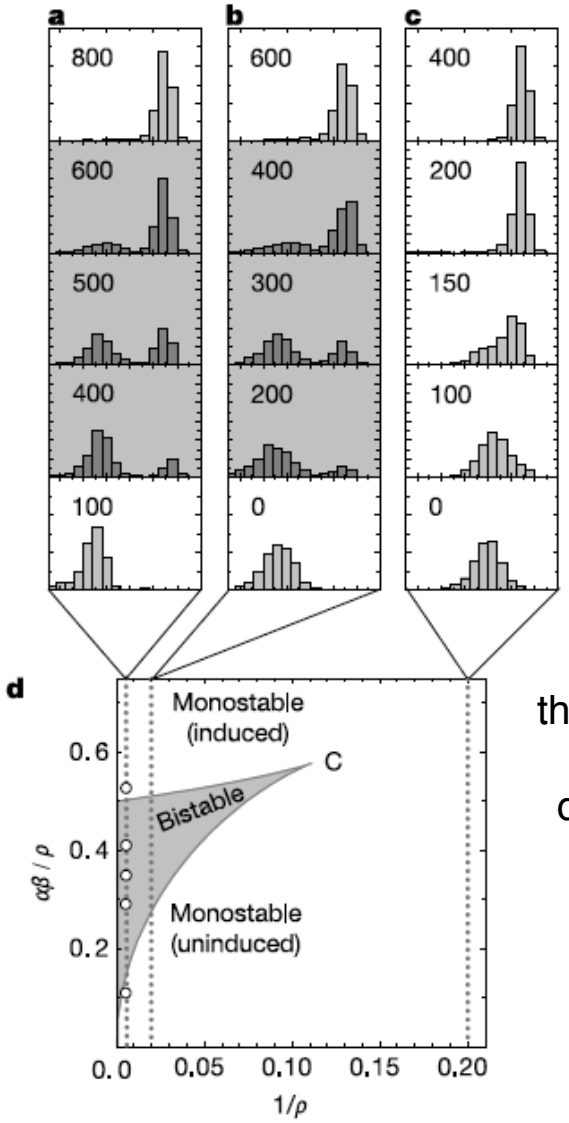




starting from uninduced cells

Multistability in the lactose utilization network of *E. coli*

Ozbudak, Thaittai, Lim, Shraiman, van Oudenaarden (2004) *Nature* 427: 737-740



theoretical phase diagram

Experiment: switch from a low (uninduced) or high (induced) TMG medium to an intermediary TMG medium (bistability)

(a) wild type — hysteretic response

(b) lacI binding site inserted in 4 plasmids — hysteretic response

(c) lacI binding site inserted in 25 plasmids — gradual response

$$\frac{R}{R_T} = \frac{1}{1+(x/x_0)^n}$$

$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1+R/R_0} - y$$

$$\tau_x \frac{dx}{dt} = \beta y - x$$

parameters values are estimated experimentally

Multistability in the lactose utilization network of *E. coli*

Ozbudak, Thaittai, Lim, Shraiman, van Oudenaarden (2004) *Nature* 427: 737-740

Conclusions

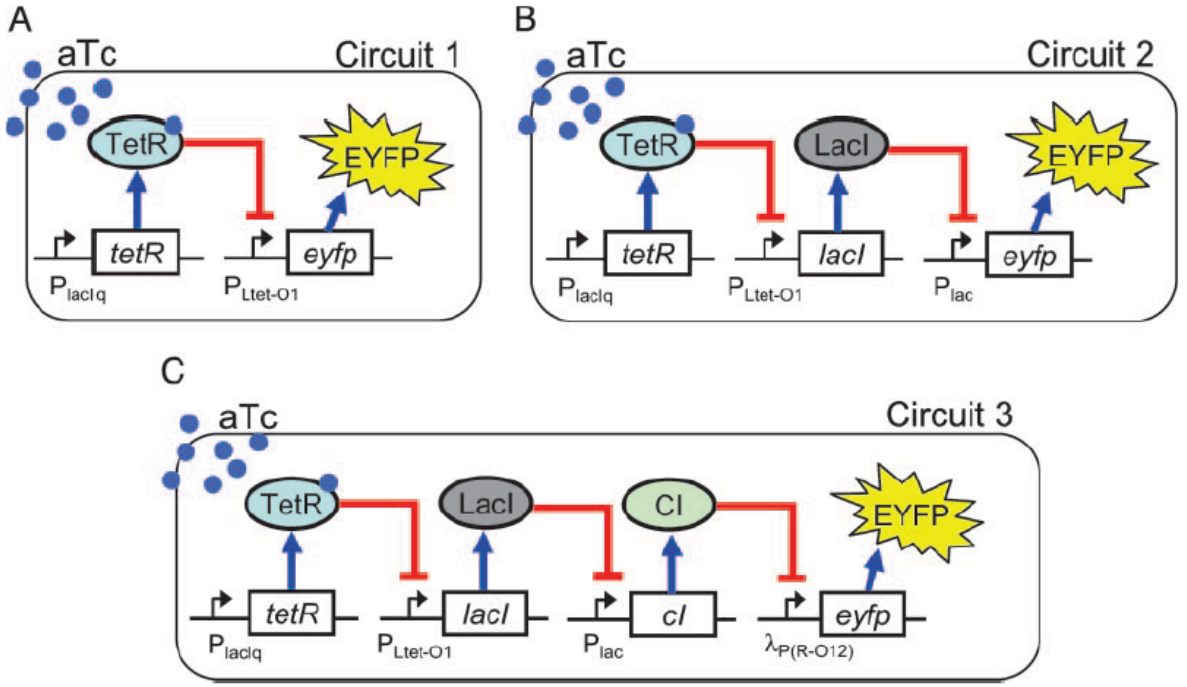
- Hysteretic vs graded responses can be achieved by modulating the parameters of the model, as predicted by a simple model.
- In the condition of hysteretic response, a bimodal distribution is observed because not all the cells switch from one steady state to the other. This is a consequence of stochastic effects.

Ultrasensitivity and noise propagation in a synthetic transcriptional cascade

Hooshangi, Thilberge, Weiss (2005) *PNAS* 102: 3581-3586

Experiment in *E. coli*:

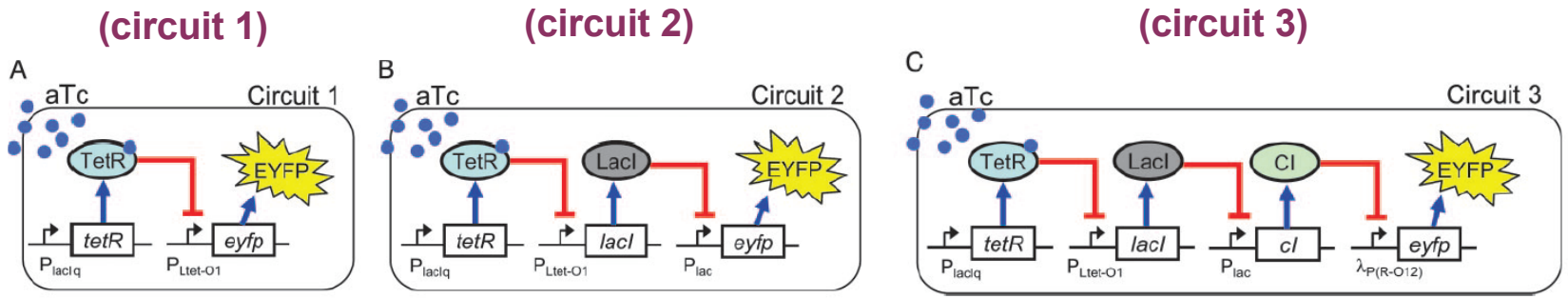
construction of 3 synthetic transcriptional cascades:

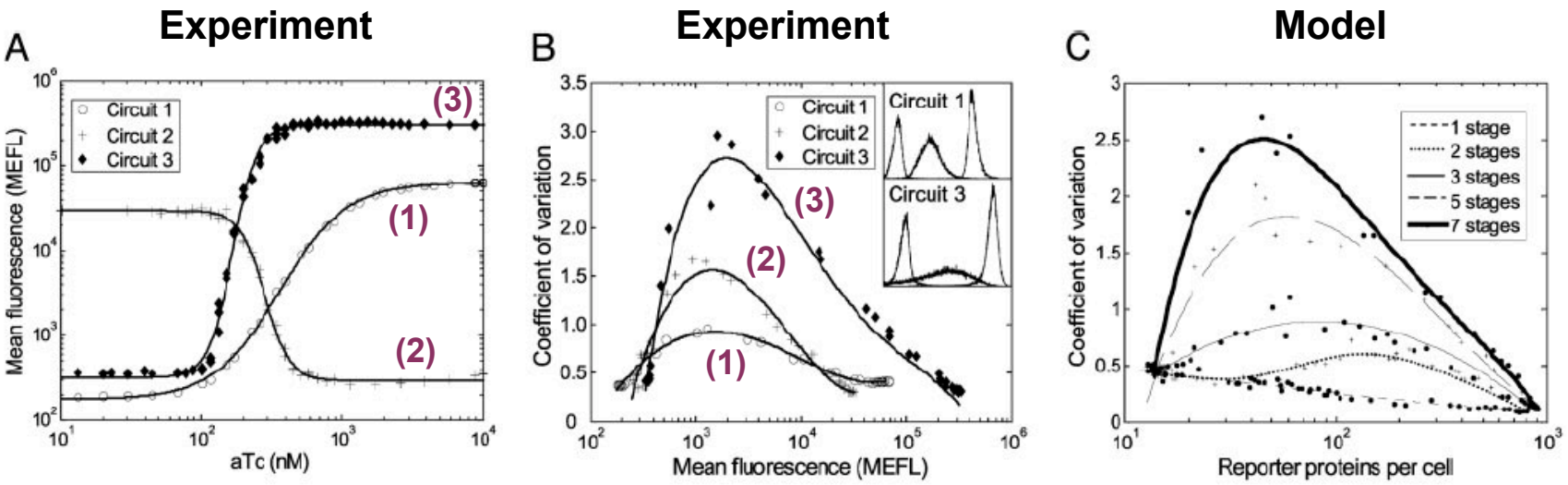


aTc = inducer = control parameter, prevent the repression by tetR

Ultrasensitivity and noise propagation in a synthetic transcriptional cascade

Hooshangi, Thilberge, Weiss (2005) *PNAS* 102: 3581-3586





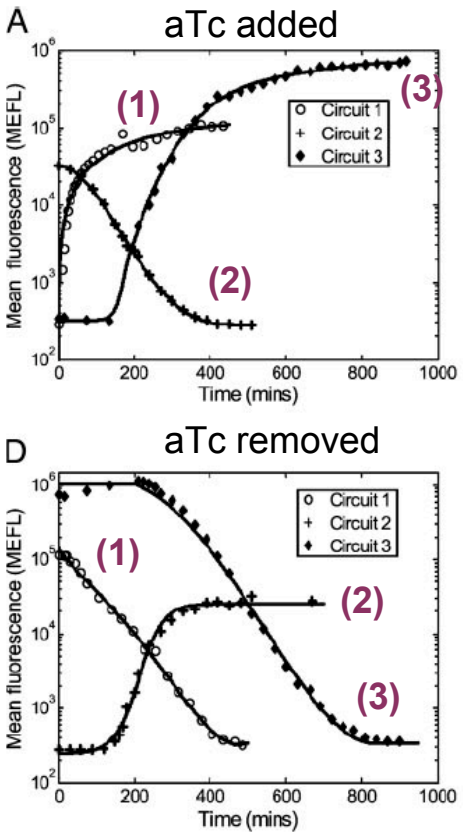
The noise is more marked during the transition, especially in circuit (3).
Longer cascade amplify cell-to-cell variability in the intermediate regions.

Ultrasensitivity and noise propagation in a synthetic transcriptional cascade

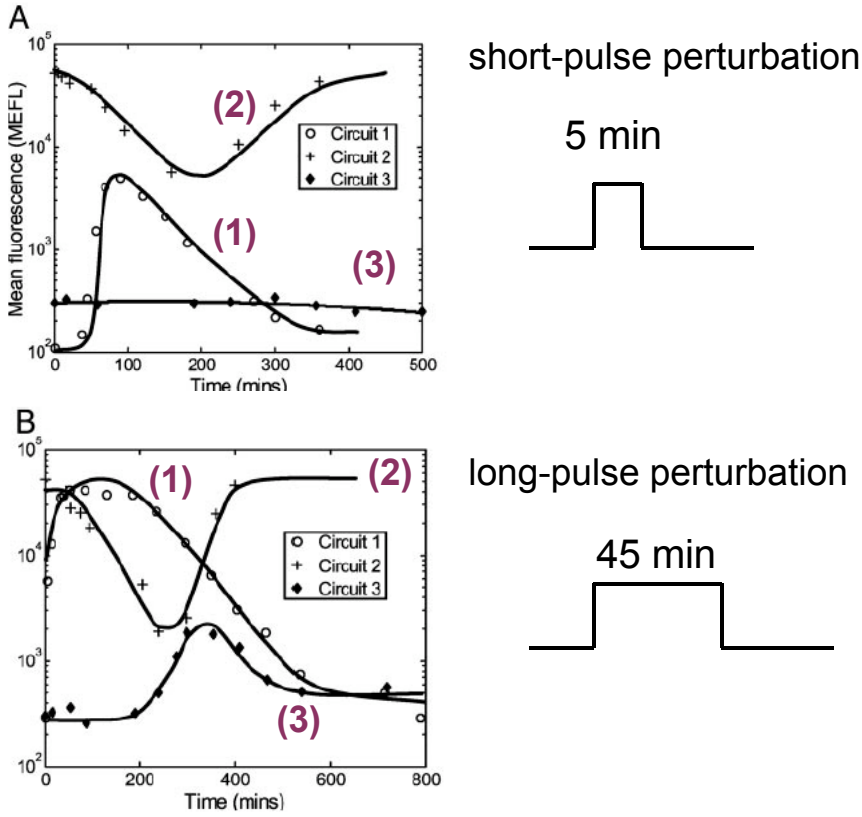
Hooshangi, Thilberge, Weiss (2005) *PNAS* 102: 3581-3586

What is the role of long cascades?

Delay in the response



Low-pass filter



short-pulse perturbation

5 min

long-pulse perturbation

45 min

Ultrasensitivity and noise propagation in a synthetic transcriptional cascade

Hooshangi, Thilberge, Weiss (2005) ***PNAS*** **102: 3581-3586**

Conclusions

- Noise (and consequently cell-to-cell variability) is amplified at transition in long cascades.
- Synchronization of cell responses is diminished for longer cascade
- Long cascade can induce delay in the response
- Long cascade act as low pass-filter

$\Rightarrow$ Trade-off between robustness to noise and function

Control of noise in genetic networks

Control of stochasticity in eukaryotic gene expression
Raser, O'Shea (2004) *Science* 304: 1811-14

Engineering stability in gene networks by autoregulation
Becskei, Serrano (2000) *Nature* 405: 590-3

Design principles of a bacterial signalling network
Kollmann, Lodvok, Bartholomé, Timmer, Sourjik (2005) *Nature* 438: 504-507

Control of stochasticity in eukaryotic gene expression

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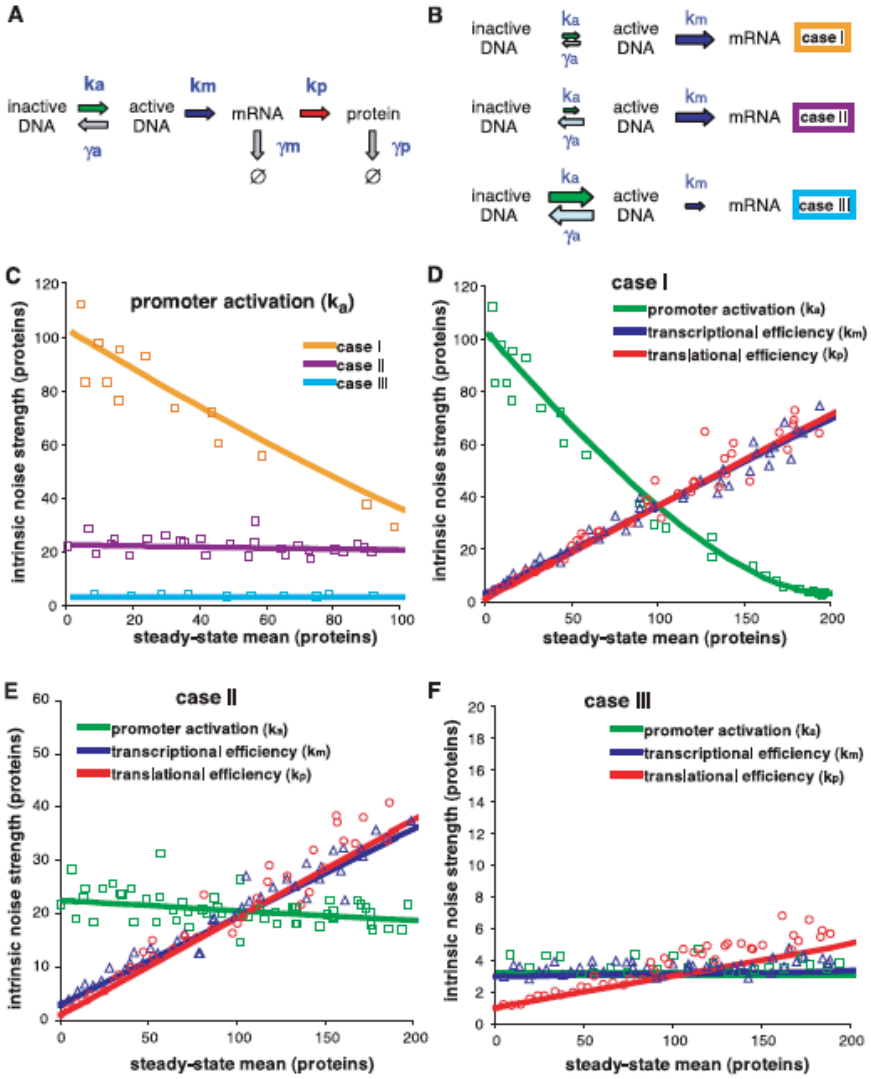


Noise can be controled by kinetics parameters

Control of stochasticity in eukaryotic gene expression

Raser, O'Shea (2004) *Science* 304: 1811-14

Conclusions

- Noise can be controled by kinetics parameters

Engineering stability in gene networks by autoregulation

Becskei, Serrano (2000) *Nature* 405: 590-3

Model and simulation

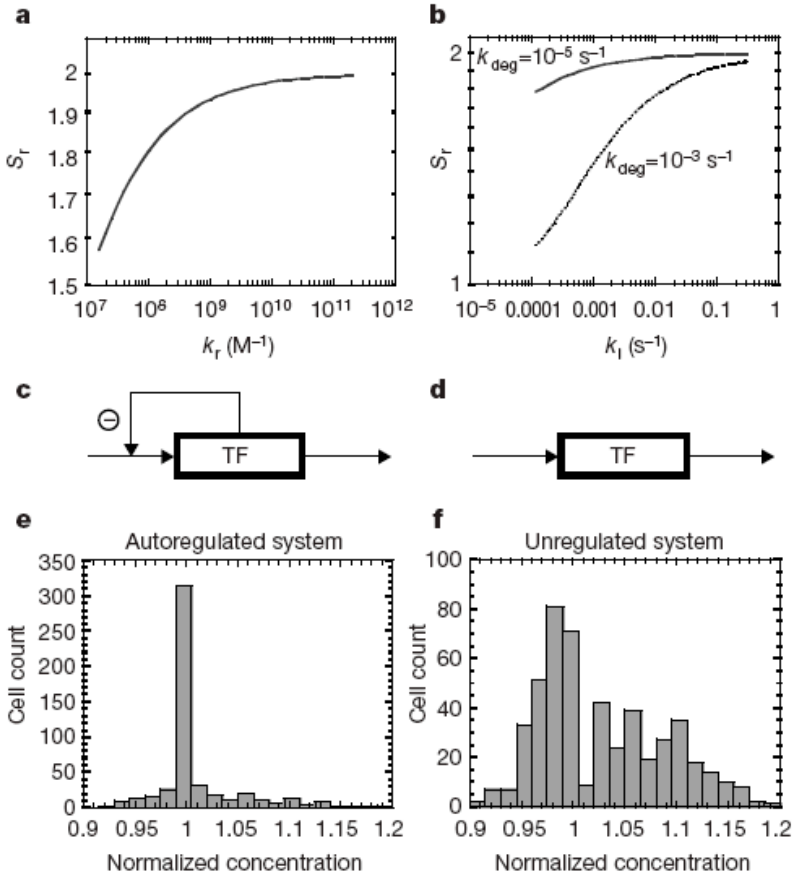
$$f_{\text{unreg}}(R) = \frac{\mathrm{d}R}{\mathrm{d}t} = n\frac{k_{\text{p}}P}{1 + k_{\text{p}}P}k_{\text{I}}a - k_{\text{deg}}R$$

$$f_{\text{auto}}(R) = \frac{\mathrm{d}R}{\mathrm{d}t} = n\frac{k_{\text{p}}P}{1 + k_{\text{p}}P + k_{\text{r}}R}k_{\text{I}}a - k_{\text{deg}}R$$

$$S_{\text{unreg}} = f'_{\text{unreg}}(R^{\star}) = -k_{\text{deg}}$$

$$S_{\text{auto}} = f'_{\text{auto}}(R^{\star}) = -\frac{nk_{\text{p}}Pk_{\text{I}}ak_{\text{r}}}{(1 + k_{\text{p}}P + k_{\text{r}}R^{\star})^2} - k_{\text{deg}}$$

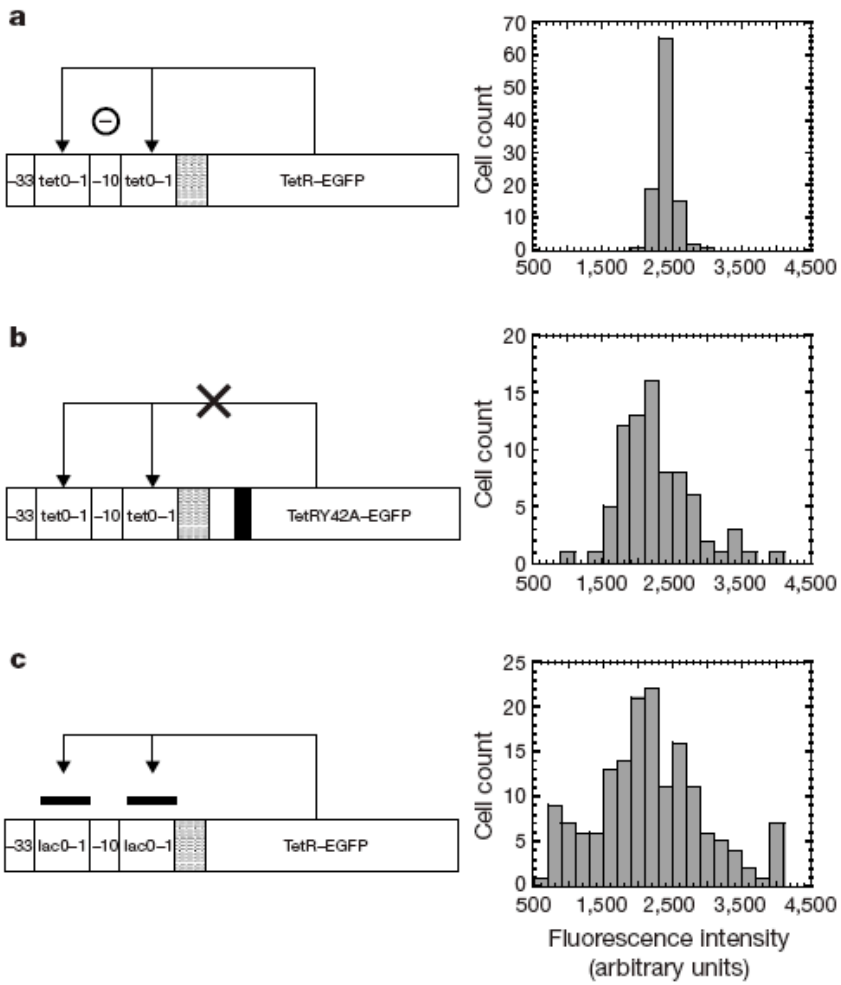
$$S_{\text{r}} = \frac{S_{\text{auto}}}{S_{\text{unreg}}}$$

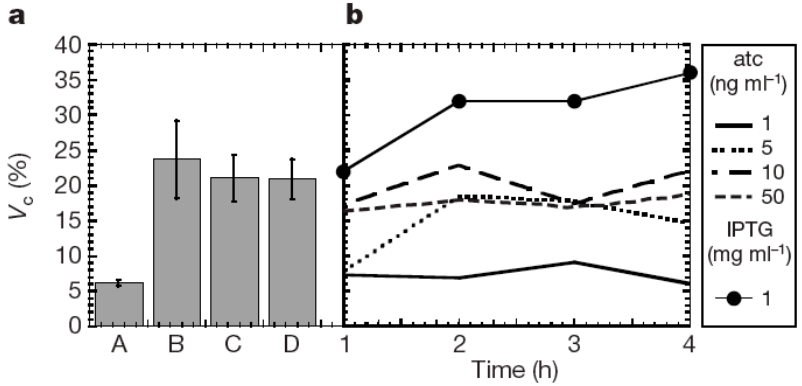


Engineering stability in gene networks by autoregulation

Becskei, Serrano (2000) *Nature* 405: 590-3

Experiment in *E. coli*





Noise can be controled by auto-regulation

Engineering stability in gene networks by autoregulation

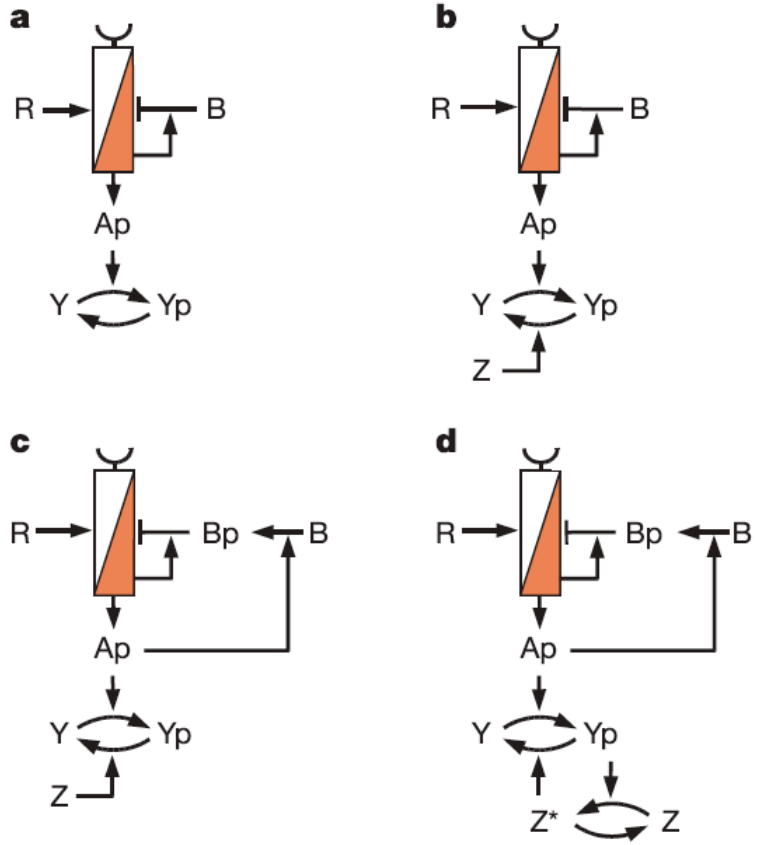
Becskei, Serrano (2000) *Nature* 405: 590-3

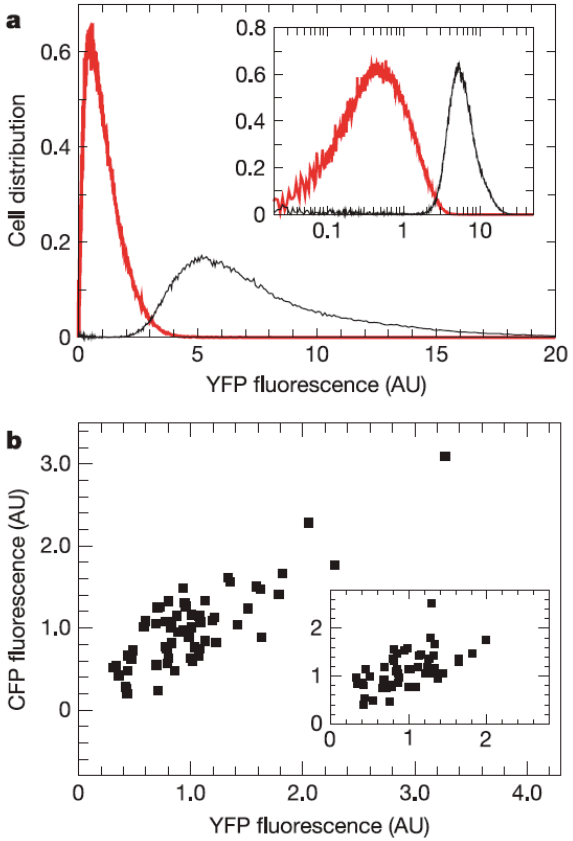
Conclusions

- Autoregulation in gene circuits (in particular negative feedback loops) provides stability.

Design principles of a bacterial signalling network

Kollmann, Lodvok, Bartholomé, Timmer, Sourjik (2005) ***Nature*** **438: 504-507**





Noise can be controled by topology of the regulatory network

Design principles of a bacterial signalling network

Kollmann, Lodvok, Bartholomé, Timmer, Sourjik (2005) *Nature* 438: 504-507

Conclusions

- Noise can be controled by topology of the regulatory network

Noise in Gene Expression: Origins, Consequences, and Control

Jonathan M. Raser[1,2] and Erin K. O'Shea[2*†]

Genetically identical cells and organisms exhibit remarkable diversity even when they have identical histories of environmental exposure. Noise, or variation, in the process of gene expression may contribute to this phenotypic variability. Recent studies suggest that this noise has multiple sources, including the stochastic or inherently random nature of the biochemical reactions of gene expression. In this review, we summarize noise terminology and comment on recent investigations into the sources, consequences, and control of noise in gene expression.

Any individual in a population of living organisms or cells is unique. Much of population variability is due to genetic differences, but environment and history also contribute to variability in cellular phenotype. Indeed, identical twin humans or cloned cats differ in appearance and behavior (Fig. 1). However, even cells or organisms with the same genes, in the same environment, with the same history, display variations in form and behavior that can be subtle or dramatic. Investigations have focused on the possibility that such variability is inevitable in biological systems because of the random nature of chemical reactions within a cell (*1*). When large numbers of molecules are present, chemical reactions may proceed in a predictable manner. However, when only a few molecules of a specific type exist in a cell, stochastic effects can become prominent.

Gene expression, as defined by the set of reactions that control the abundance of gene products, influences most aspects of cellular behavior, and its variation is often invoked to explain phenotypic differences in a population of cells. Because DNA, RNA, and proteins can be present and active at a few copies per cell, the abundance of gene products is theoretically sensitive to stochastic fluctuations. Four potential sources of variation in gene expression must be considered: (i) as described above, the inherent stochasticity of biochemical processes that are dependent on infrequent molecular events involving small

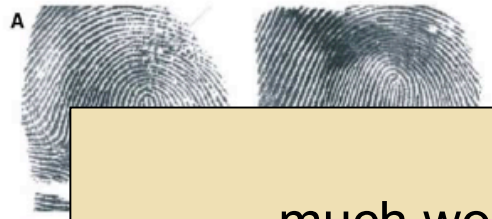


Fig. 1. Ex
fingerprin
examinati
the first c
different
Veterinary

...much work must be done to understand how cellular processes behave robustly in the presence of underlying stochasticity. Such work often requires a non-traditional collaboration between mathematicians, physicists, and *in vivo* experimentalists

[1]Medical Scientist Training Program, [2]Howard Hughes Medical Institute, University of California–San Francisco, 600 16th Street, GH-S472D, San Francisco, CA 94143-2240, USA.

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†To whom correspondence should be addressed. E-mail: erin_oshea@harvard.edu

Measurement Techniques and Definitions

Recent investigations have employed green fluorescent protein (GFP) variants, which allow the quantification of protein levels in living cells by flow cytometry or fluorescence microscopy. The coefficient of variation, or noise η, is defined as the ratio of the standard deviation to the mean of the population. Other metrics of variability can be useful as well (SOM Text).

Once genetic mutation and local microenvironments are eliminated as sources of noise, an elegant experimental method can assist in differentiating among the remaining sources

(iii) subtle environmental differences, such as morphogen gradients in multicellular development; and (iv) ongoing genetic mutation, either random or directed. We use the term "noise" in gene expression to refer to the measured level of

events in a specific signal transduction pathway. If a factor that causes extrinsic noise is experimentally manipulable, it is possible to eliminate such extrinsic noise by reduction of variability in that factor; for example, cell cycle synchronization will reduce extrinsic