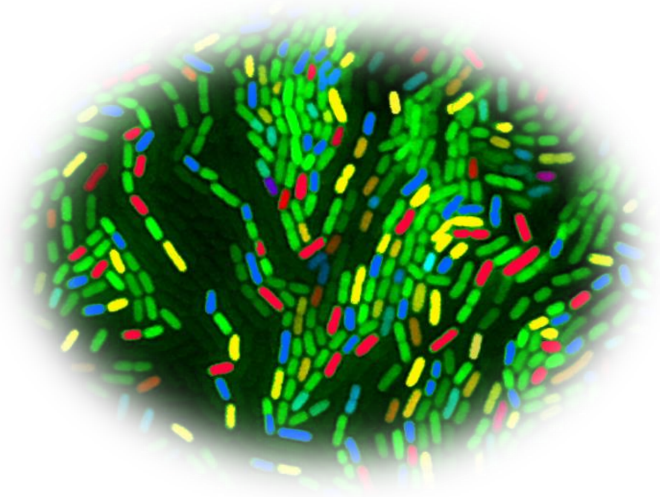
universite PARIS-SACLAY
GRADUATE SCHOOL Life Sciences & Health

Master Biologie Santé

M2 Microbiologie fondamentale

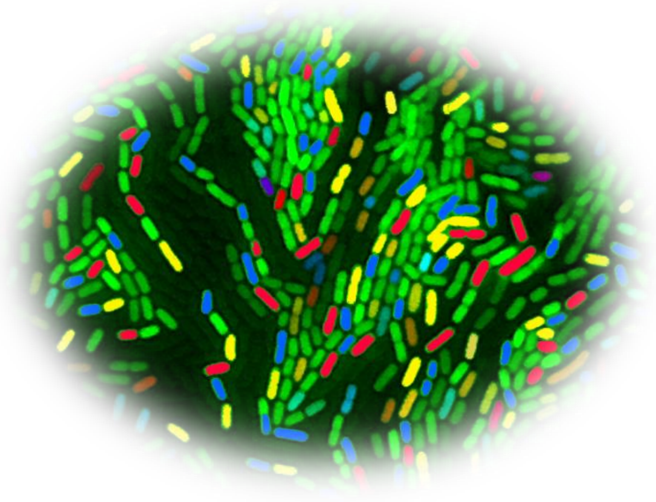
M2 Fundamental Microbiology
International Track

Stochasticity of gene expression in microorganisms, towards sociomicrobiology



Pr Stéphanie Bury-Moné
stephanie.bury-mone@universite-paris-saclay.fr
Genome biology department, I2BC

What is stochasticity?



What is stochasticity?

The notion of "noise" in Biology refers to any random and unpredictable disturbance affecting any biological phenomenon.

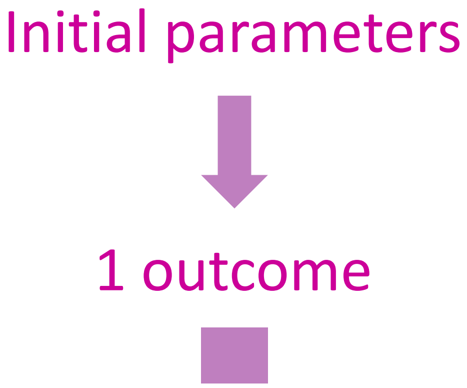
What is stochasticity?

Stochastic

Greek *Stokhastikos*, conjecturale
Stokhos, goal

Deterministic model: the outcome is fully determined by parameter values and initial conditions.

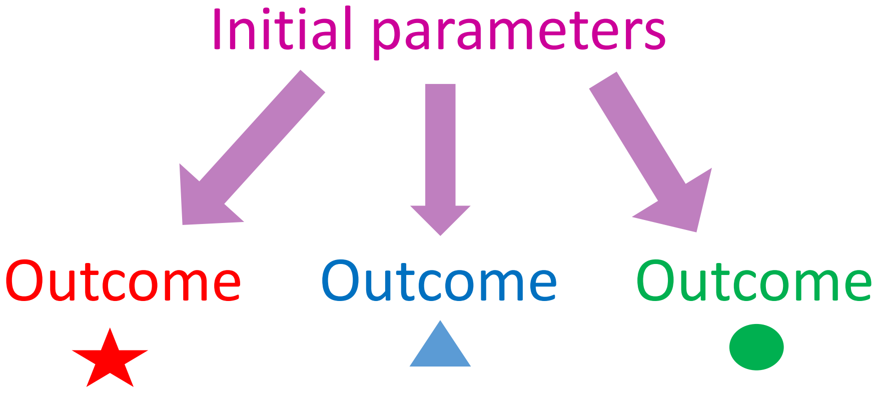
Stochastic model: "A model that, starting from the same set of initial conditions, makes it possible to predict several possible outcomes with different probabilities.”



Initial parameters

1 outcome

■



Initial parameters

Outcome ★ | Outcome ▲ | Outcome ●

=> Probabilistic model

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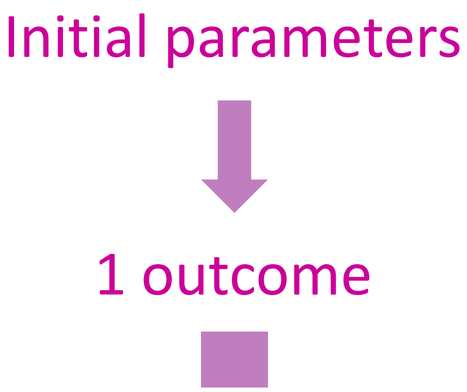

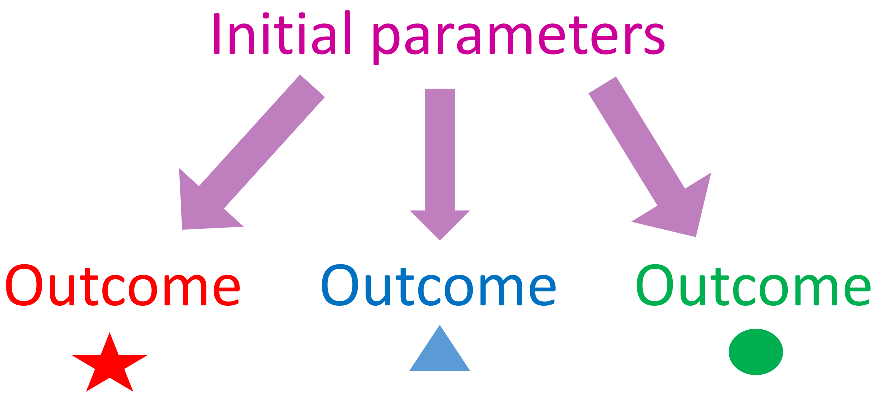

=> Probabilistic model

Is Life governed by “defined” or “probable” processes?

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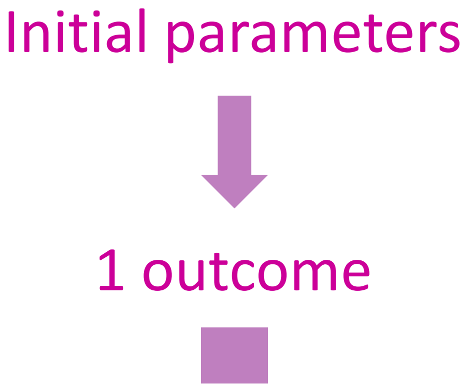
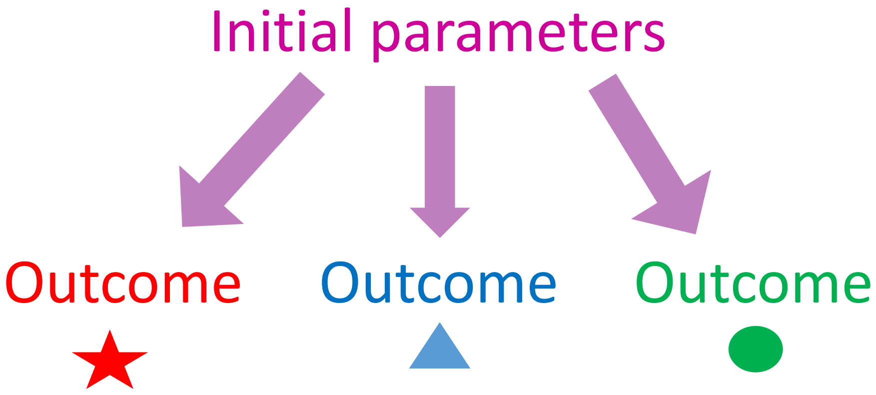

Initial parameters

1 outcome

■


Initial parameters

Outcome ★

Outcome ▲

Outcome ●

=> Probabilistic model

Are these processes really "probable", or are they simply too complex to seem "defined" to us?

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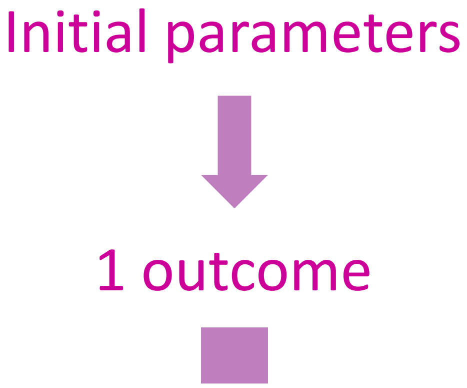
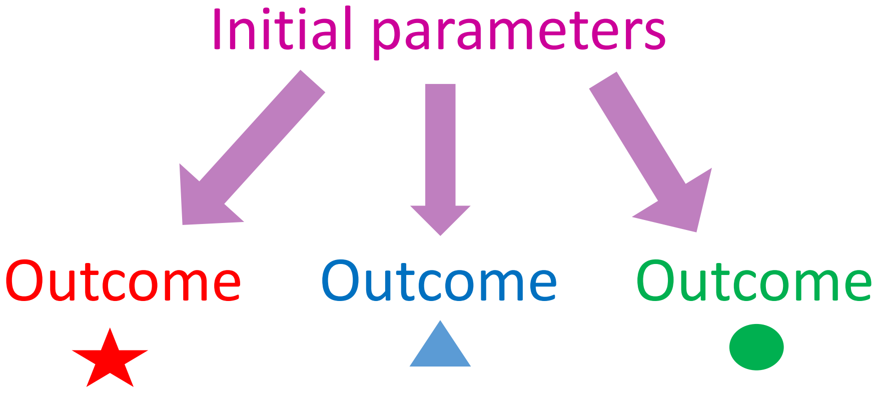


Initial parameters

1 outcome

■



Initial parameters

Outcome ★ Outcome ▲ Outcome ●

=> Probabilistic model

In practice, if there is an immense number of causes (deterministic modeling impossible), we will say that it is stochastic...

Ce que nous appelons le hasard n'est et ne peut être que la cause ignorée d'un effet connu.

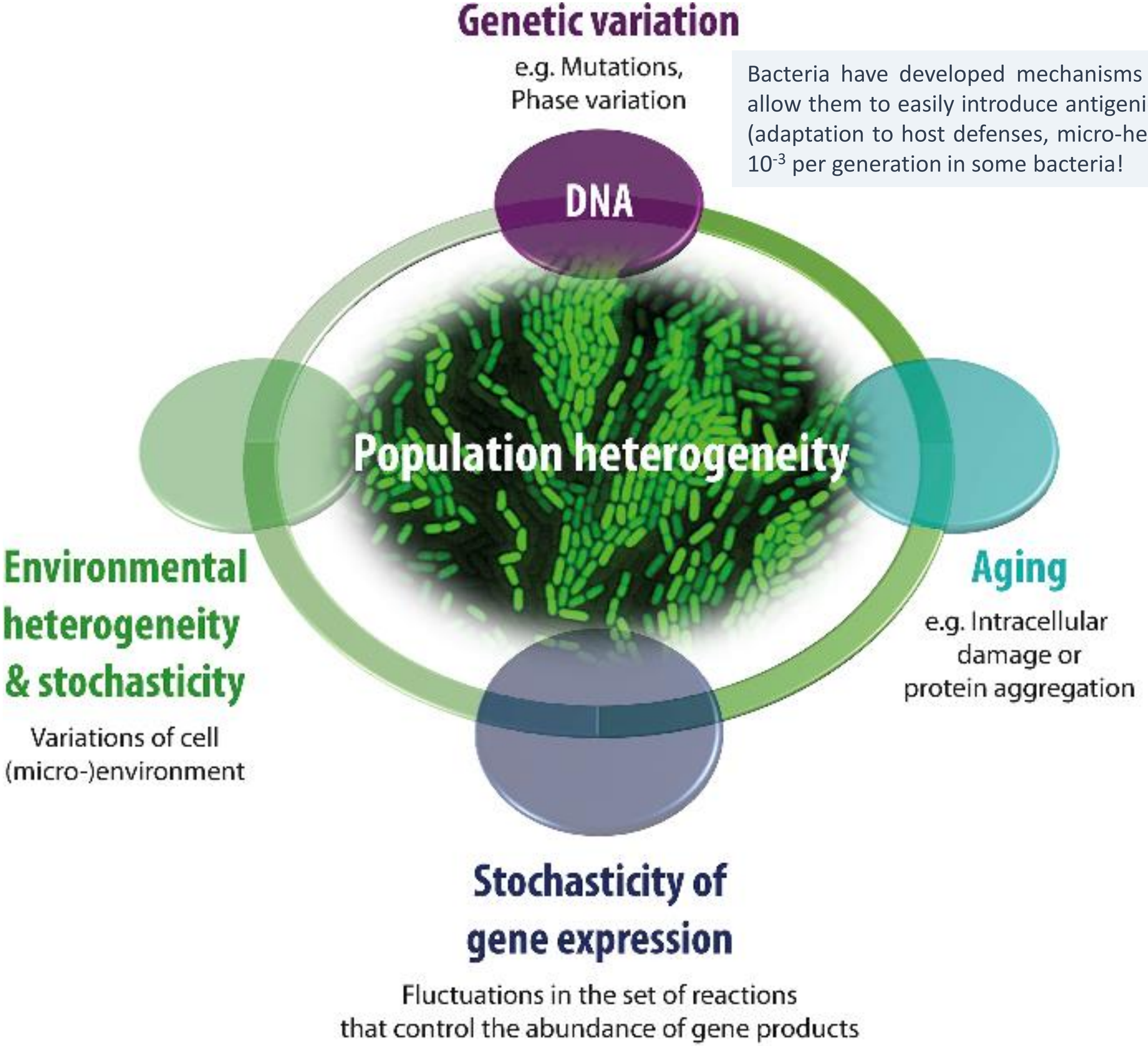
What we call random is and can only be the unknown cause of a known effect.

Voltaire, *Dictionnaire philosophique portatif* (1764)

=> Voltaire was deterministic.

Why study stochasticity?

Bacteria have developed mechanisms often called "phase variation" that allow them to easily introduce antigenic variability into surface appendages (adaptation to host defenses, micro-heterogeneity in colonies), at a rate of 10^{-3} per generation in some bacteria!



Genetic variation

e.g. Mutations,
Phase variation

DNA

Population heterogeneity

Environmental heterogeneity & stochasticity

Variations of cell
(micro-)environment

Aging

e.g. Intracellular
damage or
protein aggregation

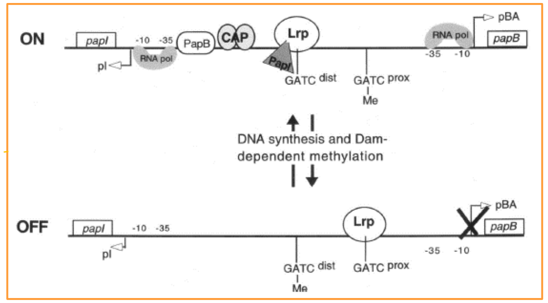
Stochasticity of gene expression

Fluctuations in the set of reactions
that control the abundance of gene products

Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

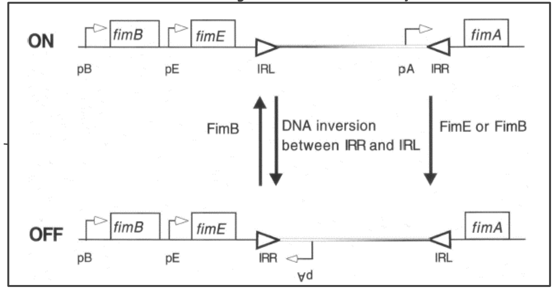
Examples of mechanisms allowing a change in surface appendages in bacteria

Ex.: Expression variation of the *E. coli pap* operon



- **Regulation of gene expression by :**
  - Transcriptional regulators
  - **DNA methylation**

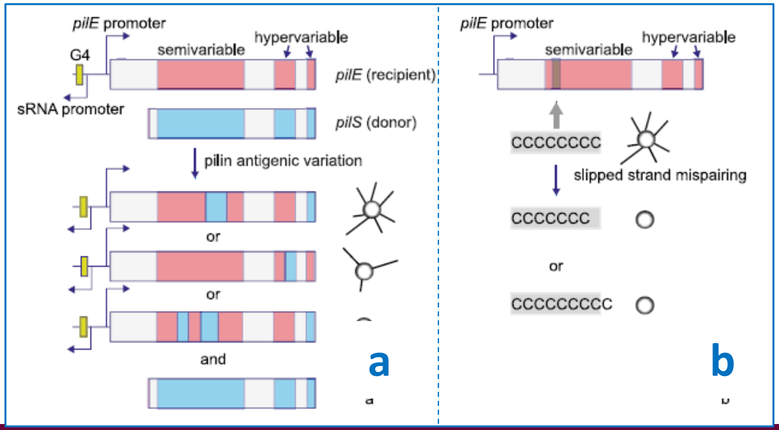
Ex. : Phase variation of type I fimbriae (FimA = major subunit) in *E. coli*



- **DNA inversion (conservative site specific recombination)**
- **Excision - integration of DNA** (e.g. IS492 at the eps locus of *Pseudoalteromonas atlantica)*
- **Gene conversion** (recombination between homologous copies)
- **Phase variation due to repetitions**

Ex.: *Neisseria gonorrhoeae* type IV pili variation by antigenic conversion (a) and phase variation related to C stretches that result in DNA polymerase slippage (b)

Zöllner *at al., Scientific Report* (2017)
van der Woude & Bäumler, *Clinical Microbiology Reviews* (2004)



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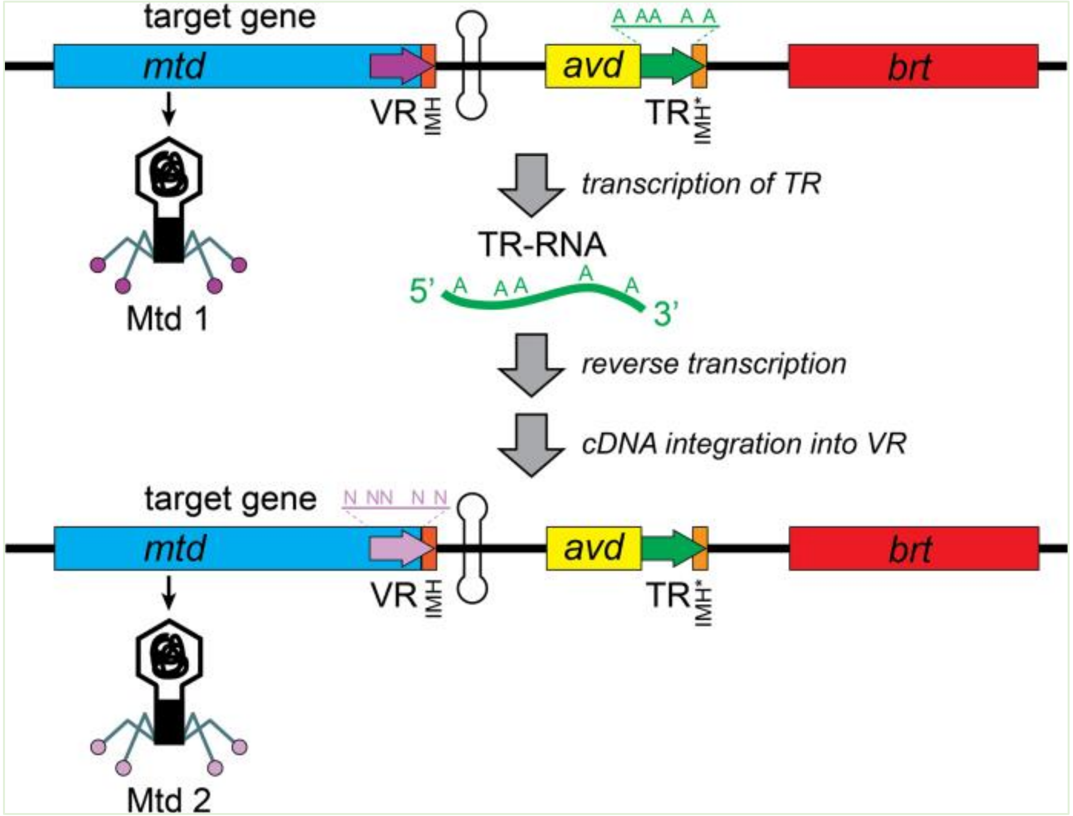
Examples of mechanisms allowing a change in the receptors in phage

DGR (Diversity-generating retroelements), a new mechanism to generate variability!

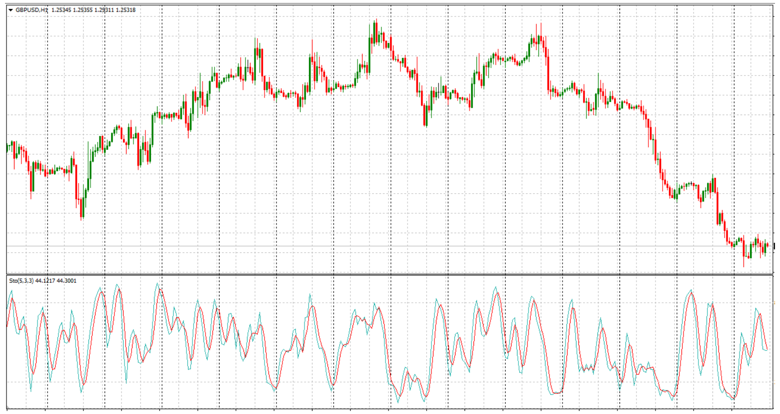
DGR mechanism (characterized in 2002) allowing the change of tropism of a *Bordetella pertussis* phage



Wu *et al., NAR* (2018)

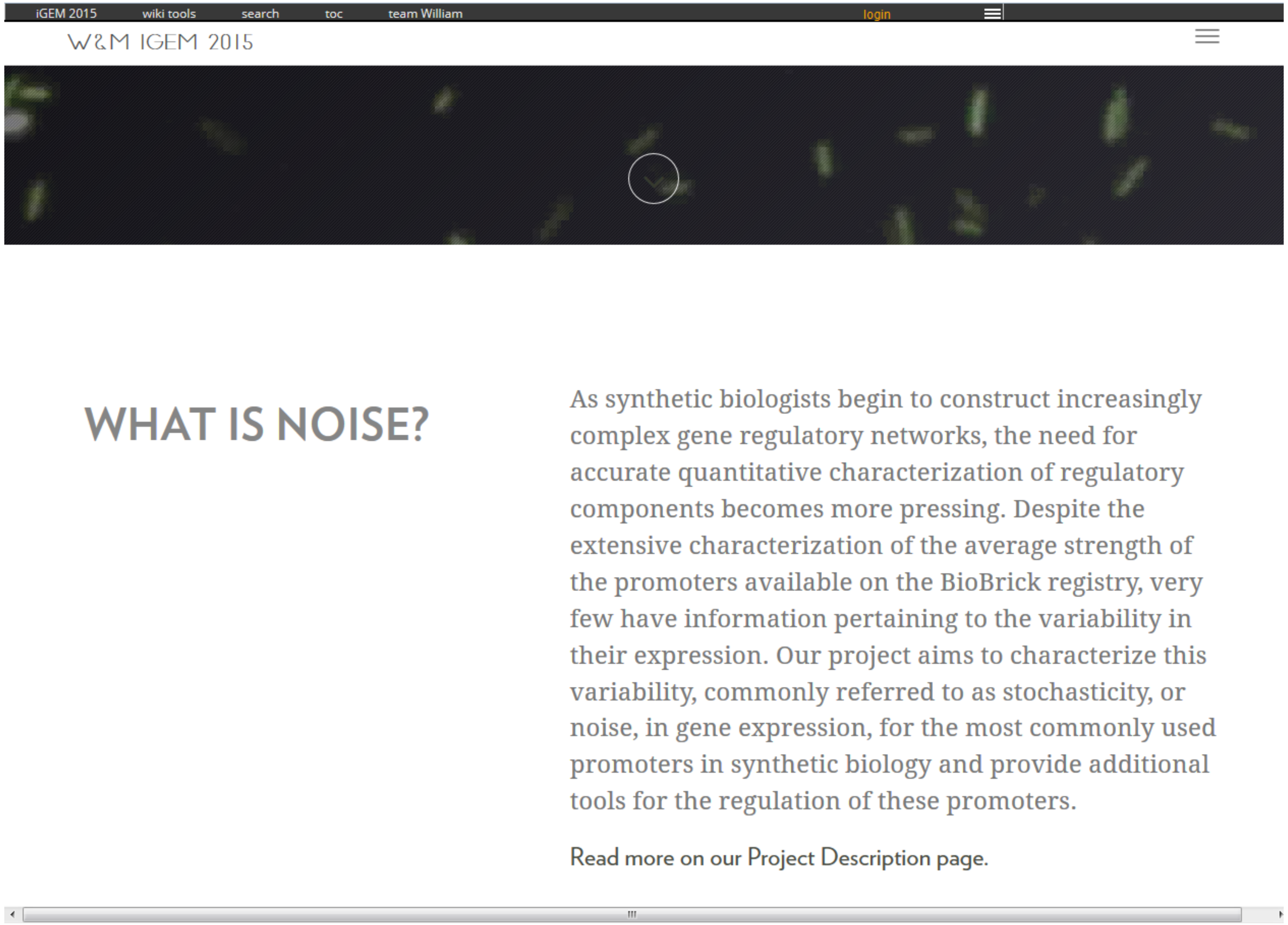
Experimentally characterized in *Bordetella* and *Legionella*, but would be quite common (not only in phage)

What is the link between stochasticity and « Cellular innovations and synthetic microbiology concepts »?



IGEM's undergraduate grand prize winning projects

http://2015.igem.org/Team:William_and_Mary

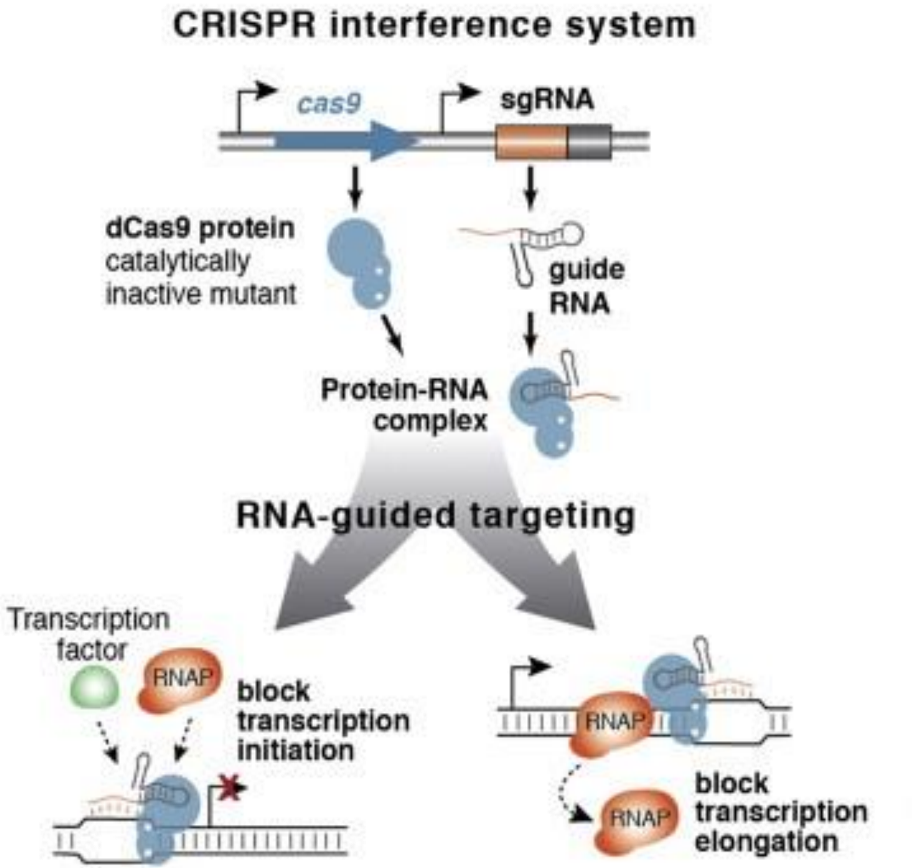


W&M IGEM 2015

WHAT IS NOISE?

As synthetic biologists begin to construct increasingly complex gene regulatory networks, the need for accurate quantitative characterization of regulatory components becomes more pressing. Despite the extensive characterization of the average strength of the promoters available on the BioBrick registry, very few have information pertaining to the variability in their expression. Our project aims to characterize this variability, commonly referred to as stochasticity, or noise, in gene expression, for the most commonly used promoters in synthetic biology and provide additional tools for the regulation of these promoters.

Read more on our Project Description page.



iGEM GO Paris-Saclay 2018

https://2018.igem.org/Team:GO_Paris-Saclay#/

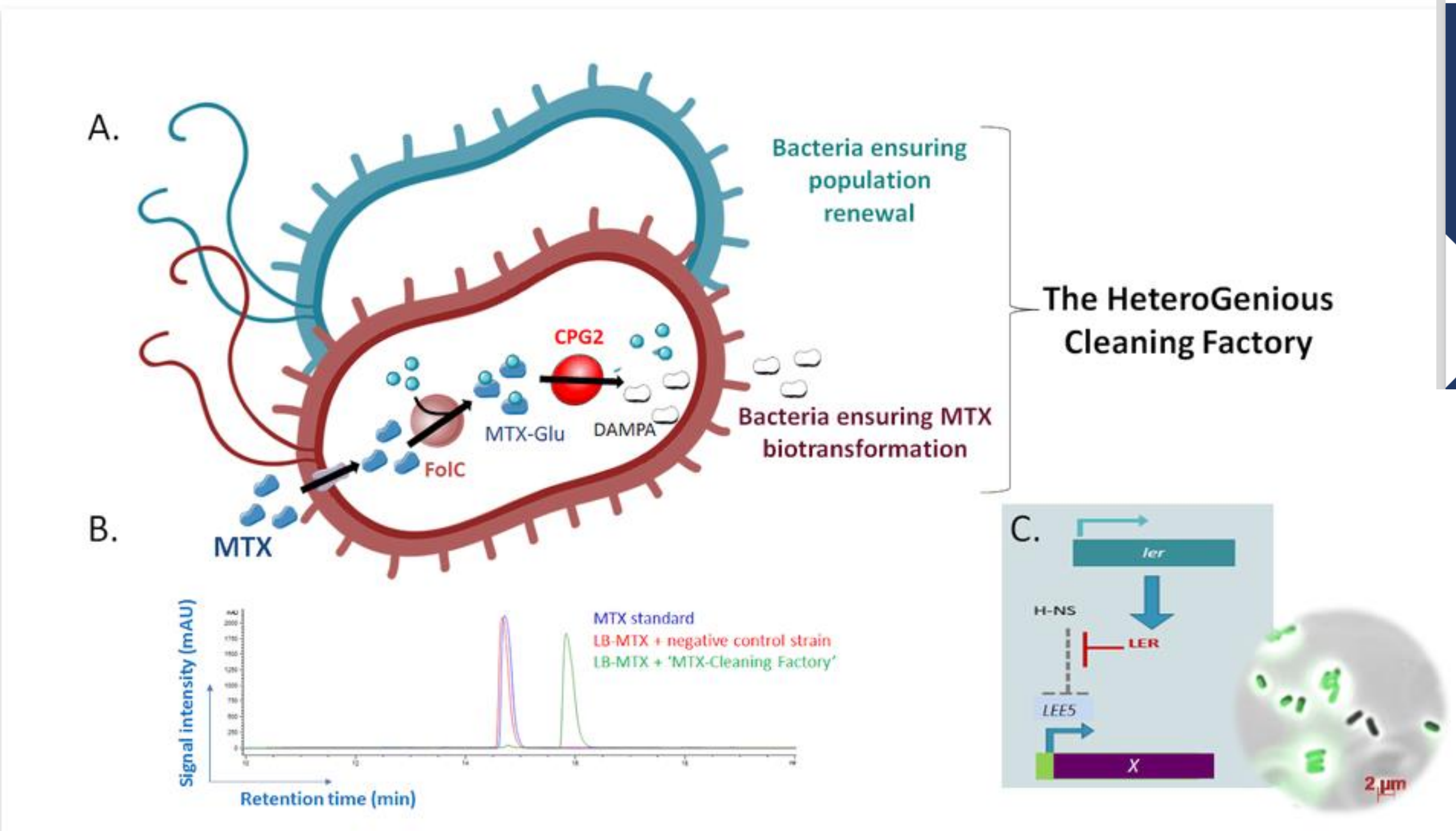


Figure 1:

A The MethotrExit project : MTX-biotransformation pathway and division of labor strategy
B HPLC analysis of MTX medium incubated with a 'MTX-Cleaning Factory'

M/S

Stochasticity of gene expression as an object of study

A Very Brief History of Stochasticity in Biology

At the end of the 60s, first uses of this notion,

- in the context of developmental noise:

Waddington CH, Kacser H "The strategy of the genes: A discussion of some aspects of theoretical biology" (1957). George Allen and Unwin, London.

- within the context of the regulation of the lactose operon:

Novick A & Weiner M, "Enzyme induction as an all-or-none phenomenon" (1957), PNAS

Publication in the 1970s of pioneering articles concerning the notion of stochasticity of gene expression in bacteria :

Spudich & Koshland, "Non-genetic individuality: chance in the single cell", **Nature** (1976)

Rigney & Schieve, "Stochastic model of linear, continuous protein synthesis in bacterial populations", **Journal of Theoritical Biology** (1977)

Berg, "A model for statistical fluctuations of protein numbers in a microbial-population", **Journal of Theoritical Biology** (1978)

Rigney, "Stochastic model of constitutive protein levels in growing and dividing bacterial cells", **Journal of Theoritical Biology** (1979)

Rigney, "Note on the kinetics and stochastics of induced protein synthesis as influenced by various models of messenger RNA degradation ", **Journal of Theoritical Biology** (1979)

Stochasticity and technological advances

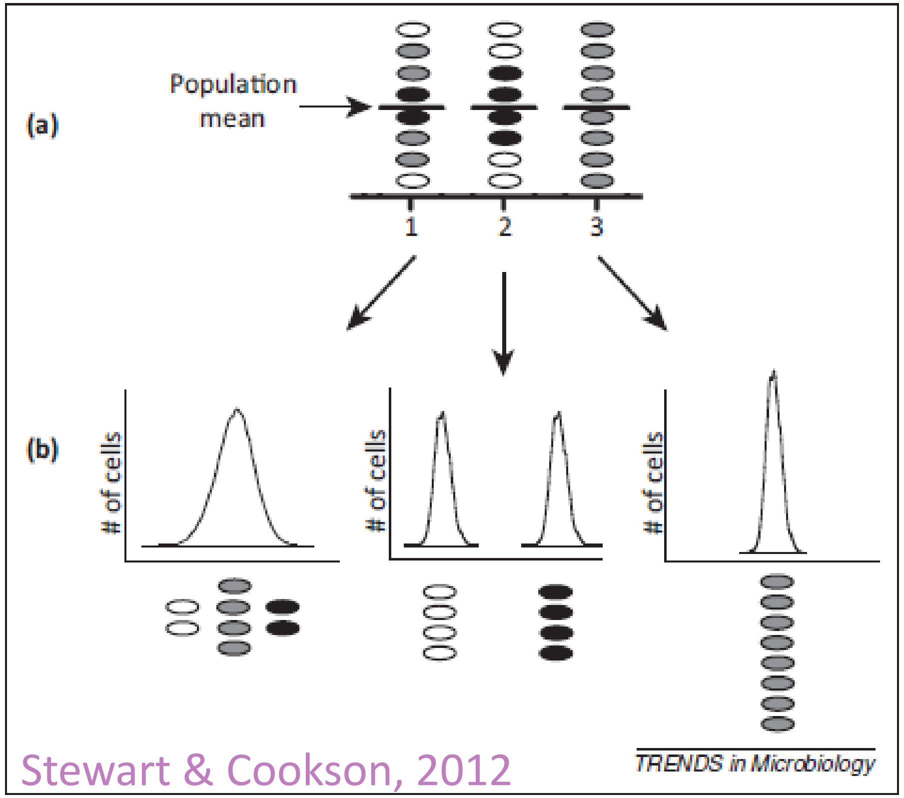
Access to the heterogeneity of cell populations

Traditional methods

(e.g. RNA or protein extraction from a biological sample, beta-galactosidase assay...) :
Measurement of a **mean** (µ)

Single cell methods:

Measurement of a phenotype of each cell
Distribution analysis
Monitoring the fate of a cell



Stewart & Cookson, 2012

Measuring the mean can pose problems of interpretation. when the differences in expression between cells span several logs...

Would you say that condition B is an ‘inducing condition’ of the reporter gene expression?

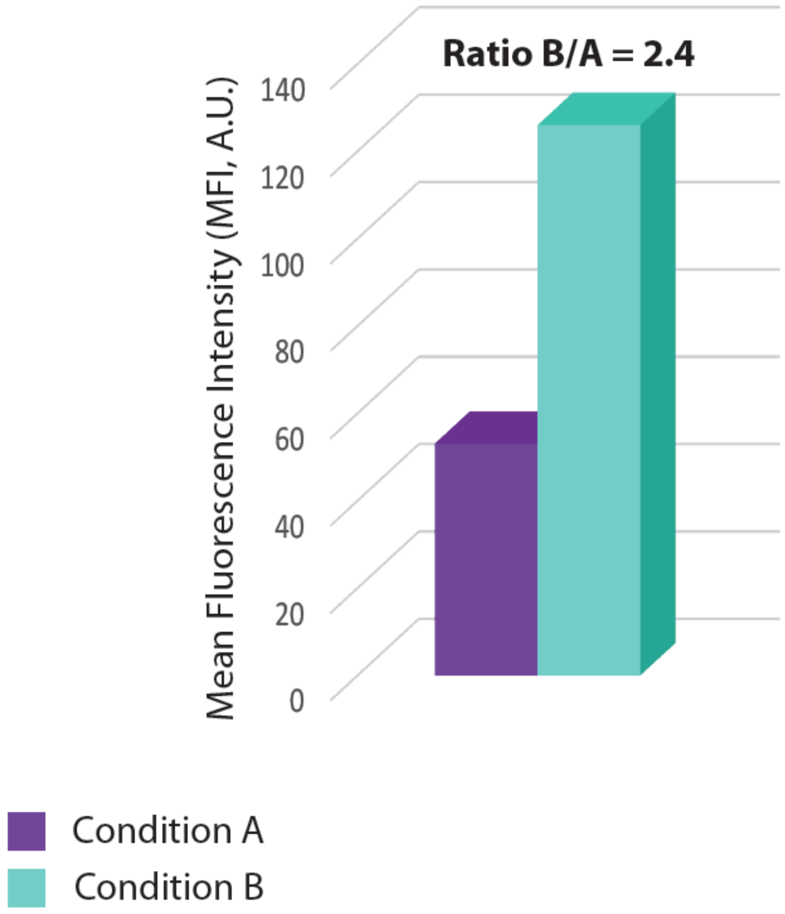
Promoter-*gfp*

Yes 👍

No 👎

Total population analysis

Averaging method



Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

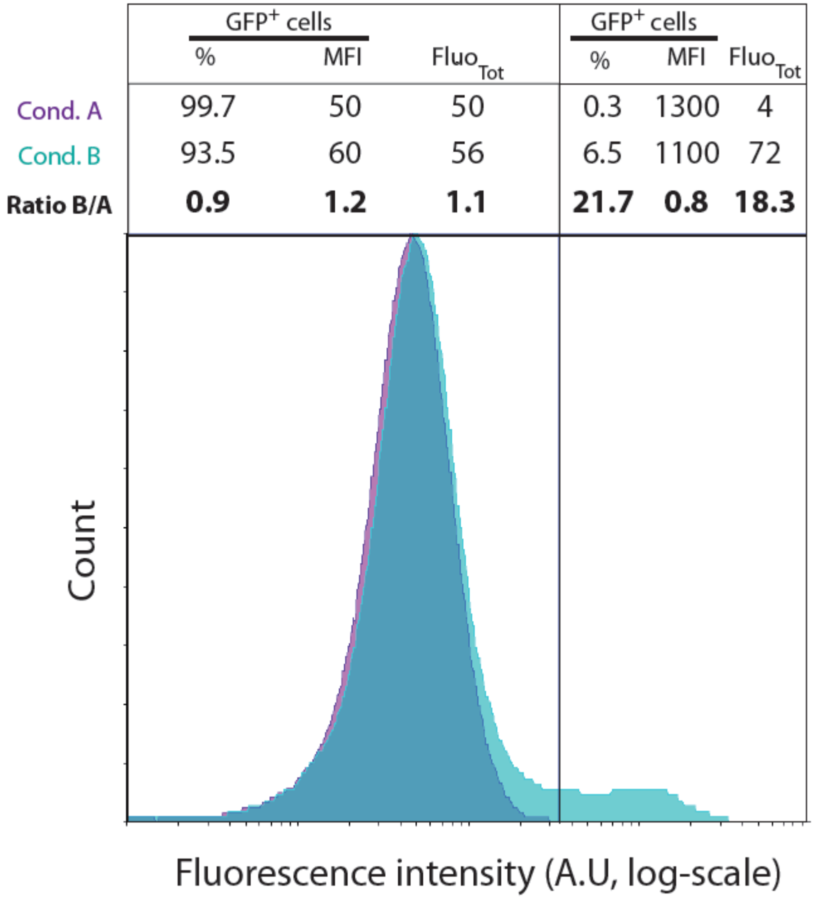
Measuring the mean can pose problems of interpretation. when the differences in expression between cells span several logs...

Promoter-*gfp*

Would you say that condition B is an 'inducing condition' of the reporter gene expression?

Yes **No**

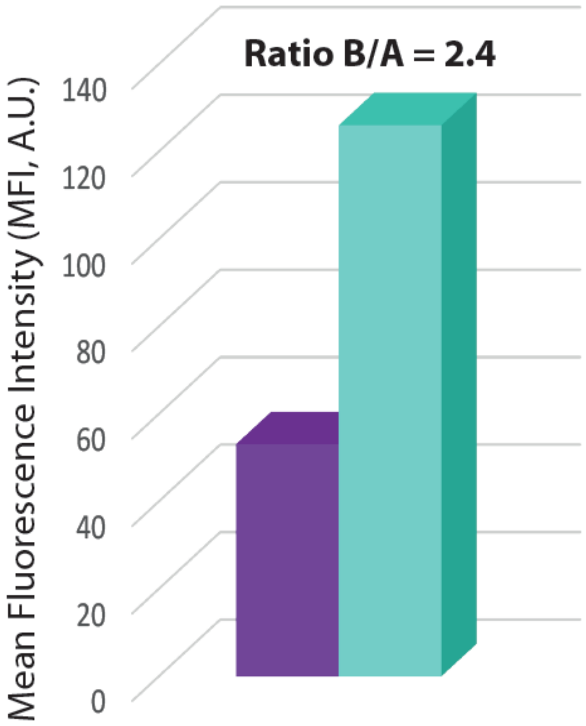
Single cell analysis



	GFP$^+$ cells			GFP$^+$ cells		
	%	MFI	Fluo$_{Tot}$	%	MFI	Fluo$_{Tot}$
Cond. A	99.7	50	50	0.3	1300	4
Cond. B	93.5	60	56	6.5	1100	72
Ratio B/A	**0.9**	**1.2**	**1.1**	**21.7**	**0.8**	**18.3**

Total population analysis

Averaging method



🟪 Condition A
🟩 Condition B

Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

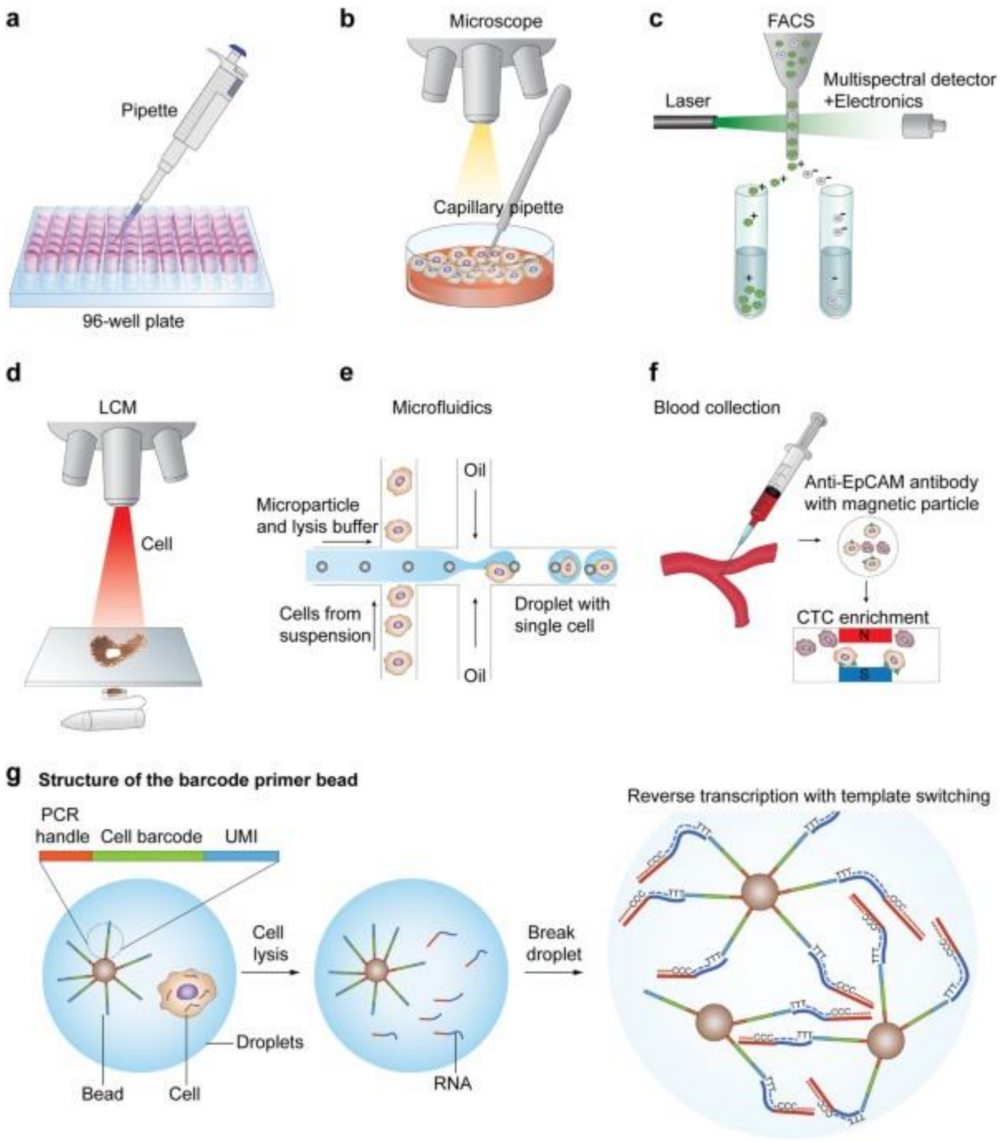
Stochasticity and technological advances

Main methodologies for **individual monitoring** and/or **isolation of cells:**

Fluorescence microscopy
Flow cytometry
Microfluidic droplets
Serial dilutions, Optical tweezers, micromanipulations, laser microdissections, laser tag activation, etc...

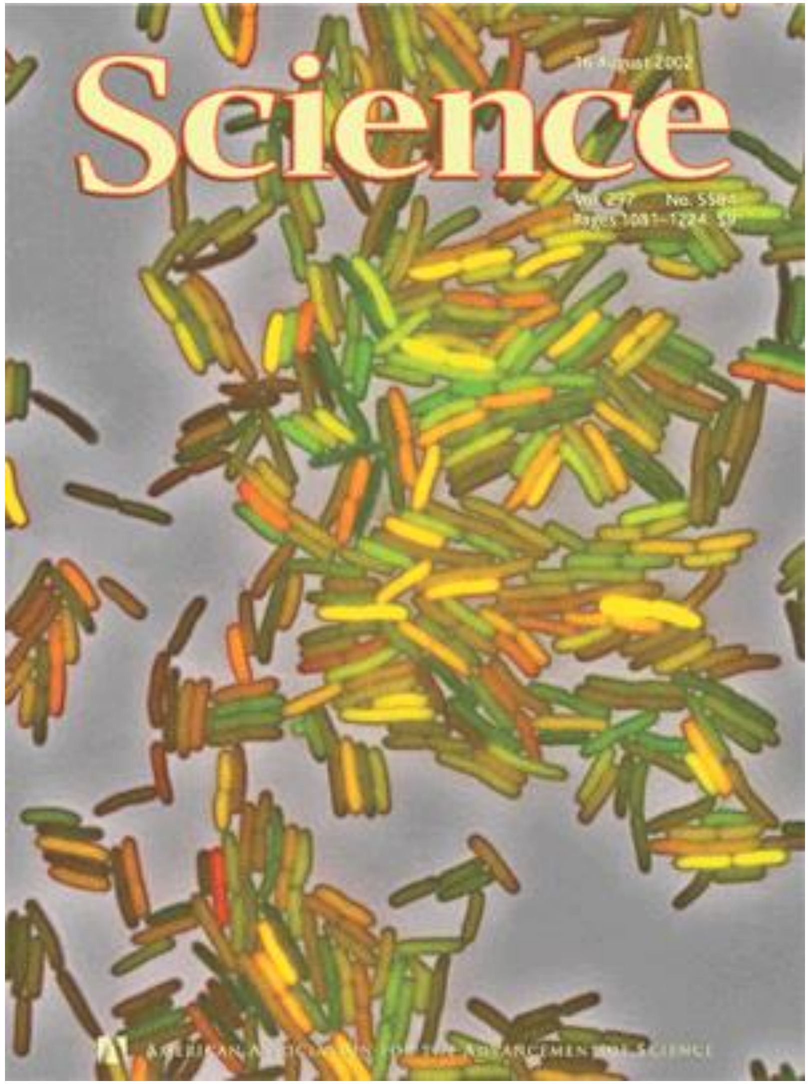
Combined with methodologies to:

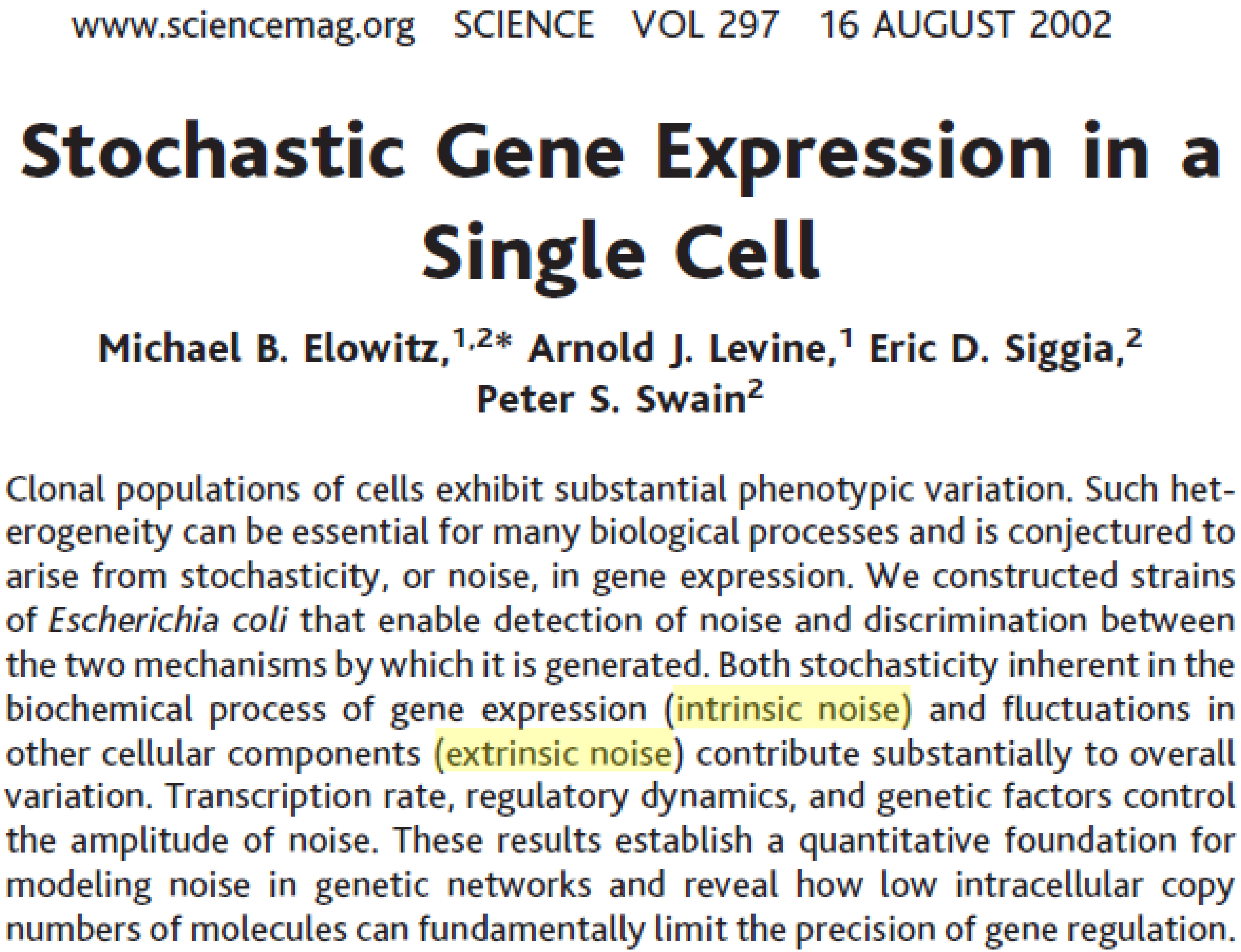
- Detect a marker (usually fluorescent) - Ex: *reporter gene,* fluorescent antibodies…
- Detecting an amplification product [*e.g.* PCR *single cell*, RT-PCR *single cell*, MDA *single cell (multiple Displacement Amplification* – Phi29 polymerase)]
    - In a targeted way (on a gene of interest)
    - In a global way – Ex: DNAseq *single cell,* RNAseq *single cell, microarrays*



Nature

The revival of the concept of stochasticity of gene expression




www.sciencemag.org SCIENCE VOL 297 16 AUGUST 2002

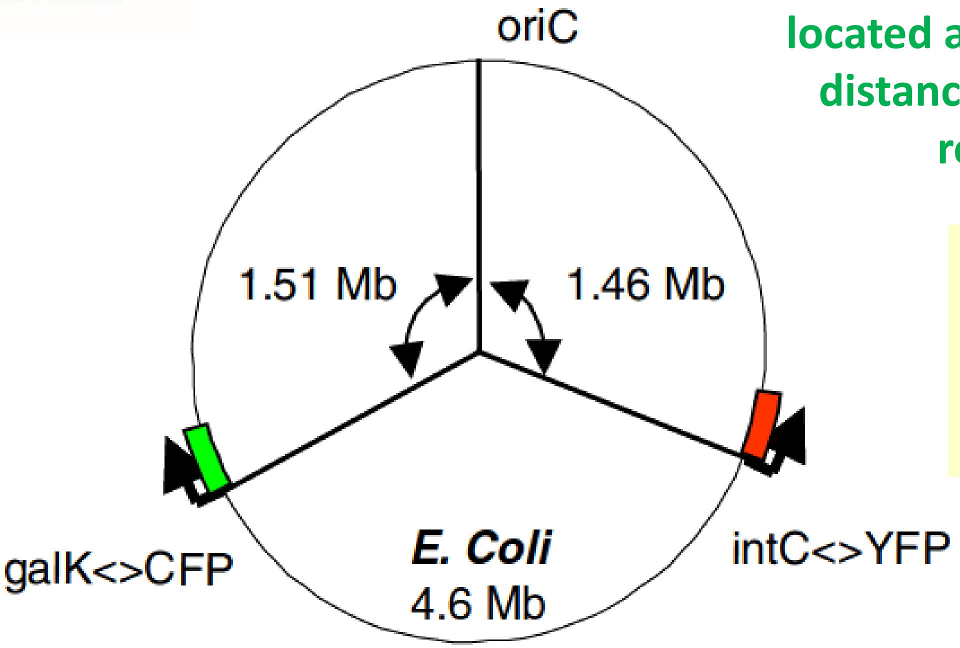
Stochastic Gene Expression in a Single Cell

Michael B. Elowitz,[1,2]* Arnold J. Levine,[1] Eric D. Siggia,[2]
Peter S. Swain[2]

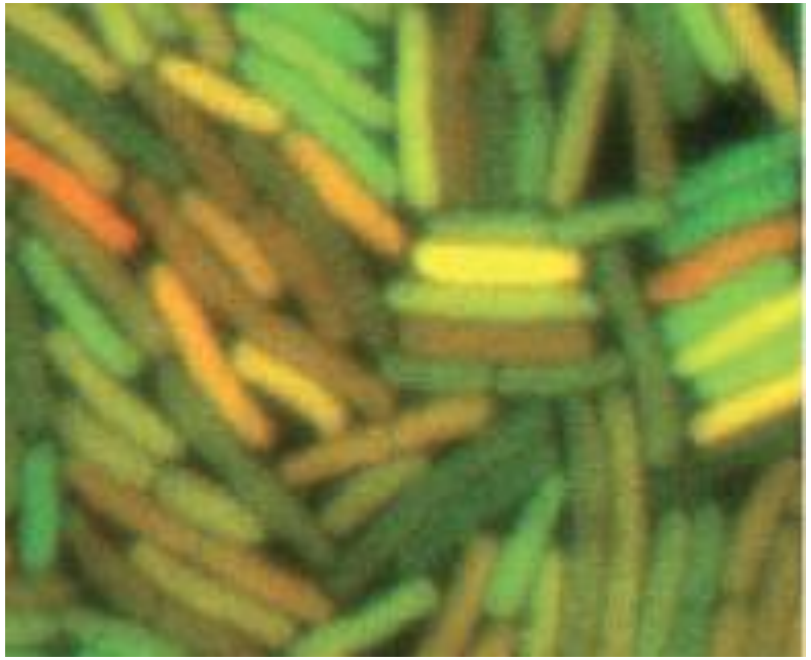
Clonal populations of cells exhibit substantial phenotypic variation. Such heterogeneity can be essential for many biological processes and is conjectured to arise from stochasticity, or noise, in gene expression. We constructed strains of *Escherichia coli* that enable detection of noise and discrimination between the two mechanisms by which it is generated. Both stochasticity inherent in the biochemical process of gene expression (intrinsic noise) and fluctuations in other cellular components (extrinsic noise) contribute substantially to overall variation. Transcription rate, regulatory dynamics, and genetic factors control the amplitude of noise. These results establish a quantitative foundation for modeling noise in genetic networks and reveal how low intracellular copy numbers of molecules can fundamentally limit the precision of gene regulation.

Elowitz *et al.*, « Stochastic gene expression in a single cell », *Science* (2002)

Why were the two reporters located at almost the same distance of the origin of replication?



Transgene expression is under the control of the same *lac* promoter.



we built strains of *Escherichia coli*, incorporating the distinguishable cyan (*cfp*) and yellow (*yfp*) alleles of green fluorescent protein in the chromosome. In each strain, the two reporter genes were controlled by identical promoters. To avoid systematic differences in copy number, we integrated the genes at loci equidistant from, and on opposite sides of, the origin of replication

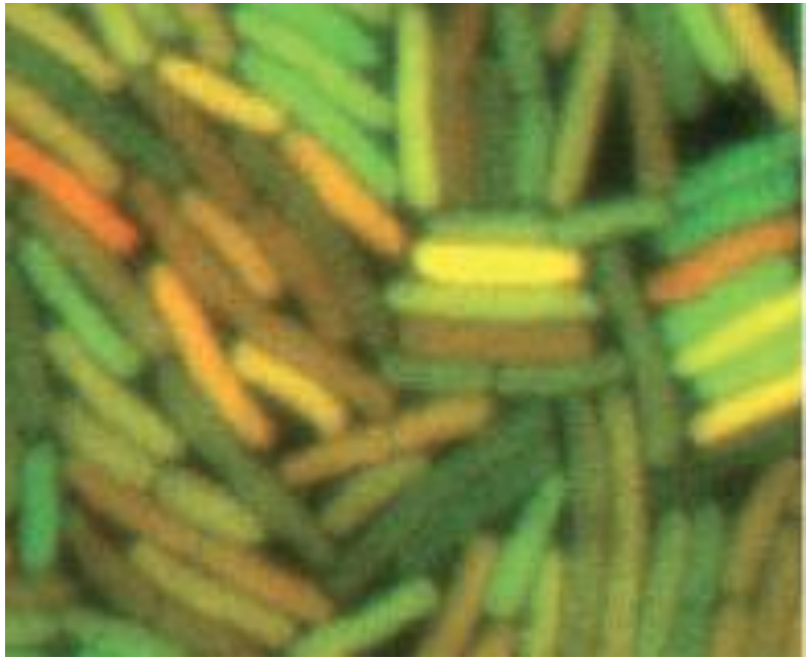
For measurement, cells were grown in LB medium and photographed through *cfp* and *yfp* fluorescence filter sets and in phase contrast (Fig. 2) (7). A computerized image analysis system identified cells and quantified their mean fluorescent intensities

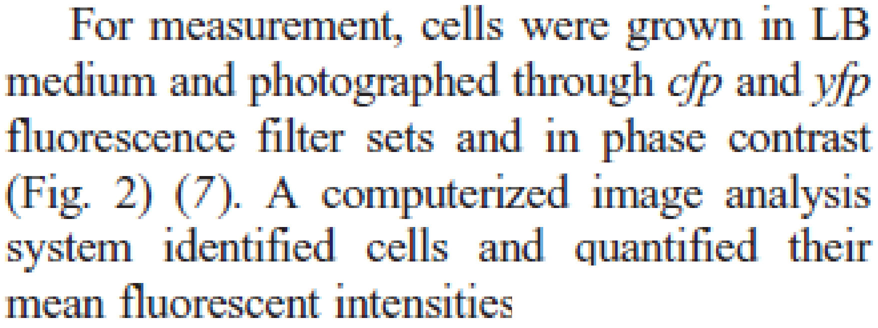
A result is represented: expression of the CFP (cyan fluorescent protein) in green, the YFP (yellow fluorescent protein) in red, both simultaneously in yellow.

Elowitz *et al., Science* (2002)

Elowitz *et al.*, « Stochastic gene expression in a single cell », *Science* (2002)

Genetically identical individuals (isogenic - clones) located in an <u>apparently homogeneous and constant</u> environment present different phenotypes.





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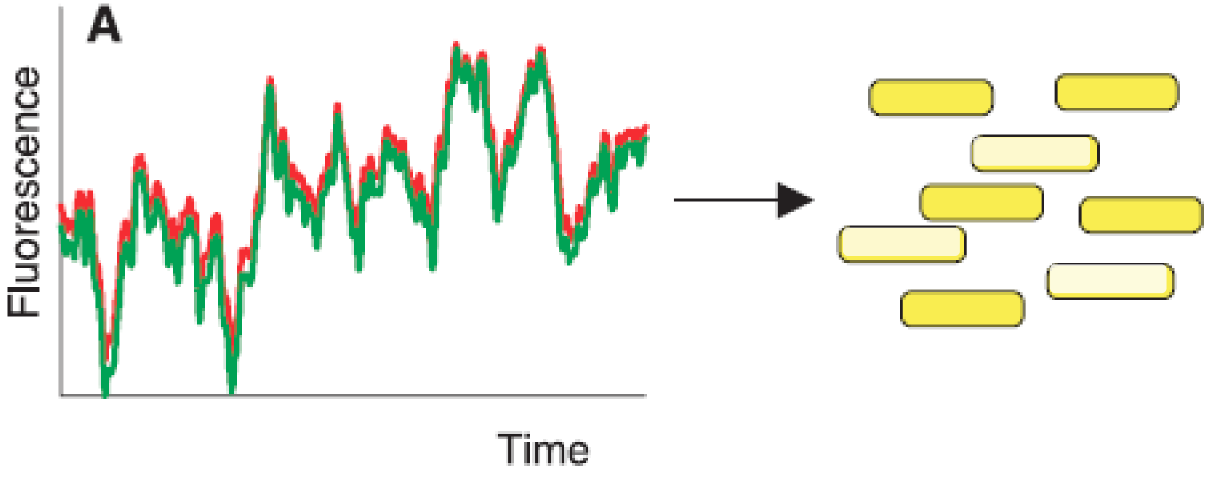
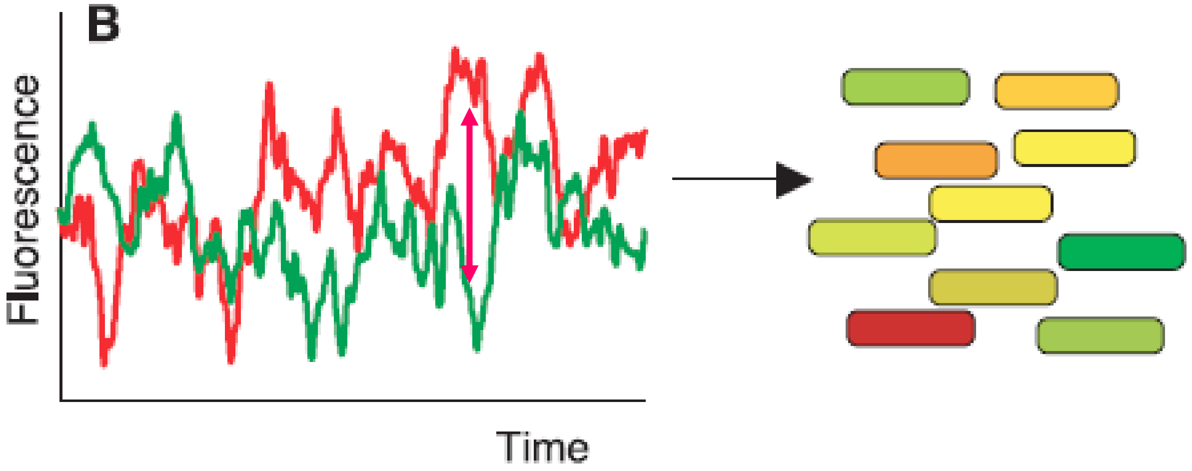
Elowitz *et al.*, « Stochastic gene expression in a single cell », *Science* (2002)

Theoretical cases of fluorescence fluctuations of the proteins YFP (red) and CFP (green) within an individual cell.

What could be the cause of the variations observed in A?



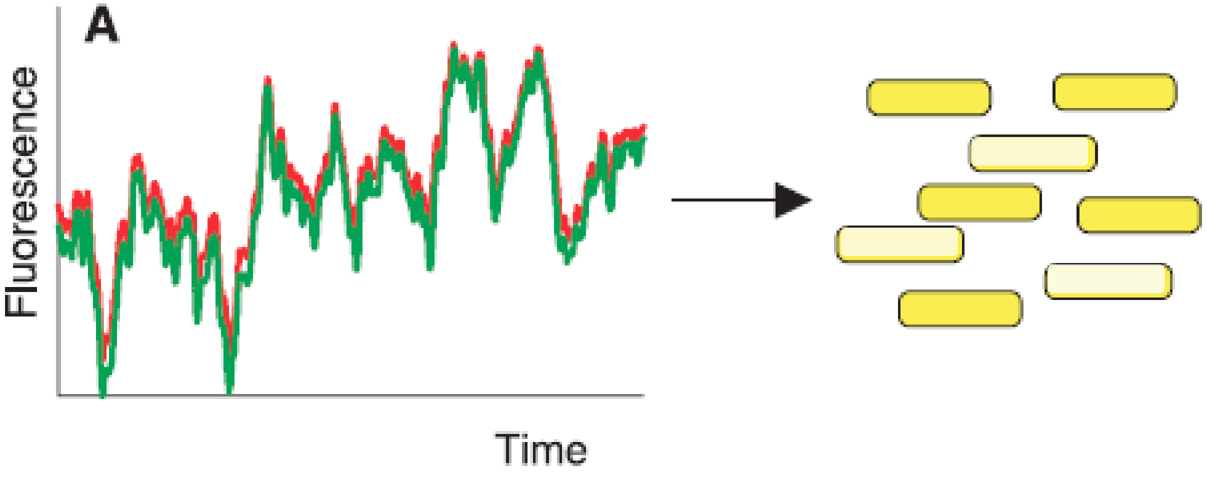


Elowitz *et al., Science* (2002)

Noise Components

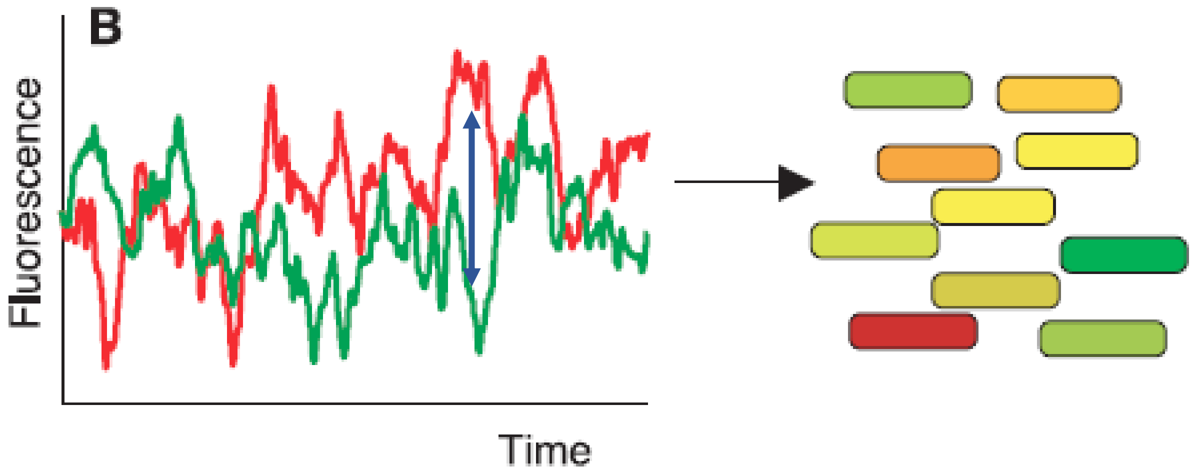
Variations in parameters modulating promoter activity and gene expression (post-transcriptional regulations) identically on both promoters.
Difference between cells or over time but not within the cell itself at a given time.

=> Extrinsic noise



Heterogeneity between cells
or of a cell over time

What additional phenomenon would be added to panel B?



Elowitz *et al.*, *Science* (2002)

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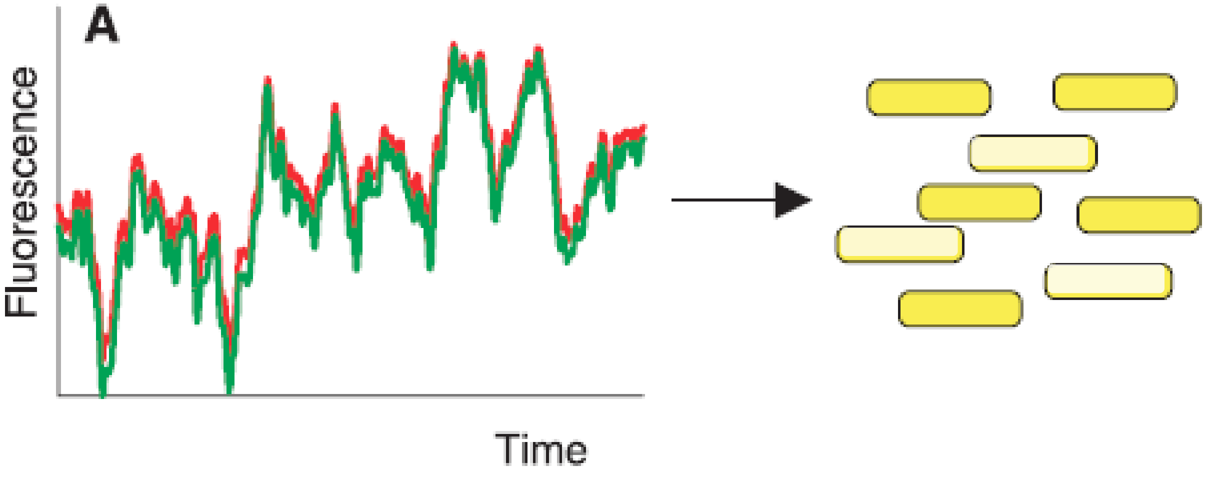
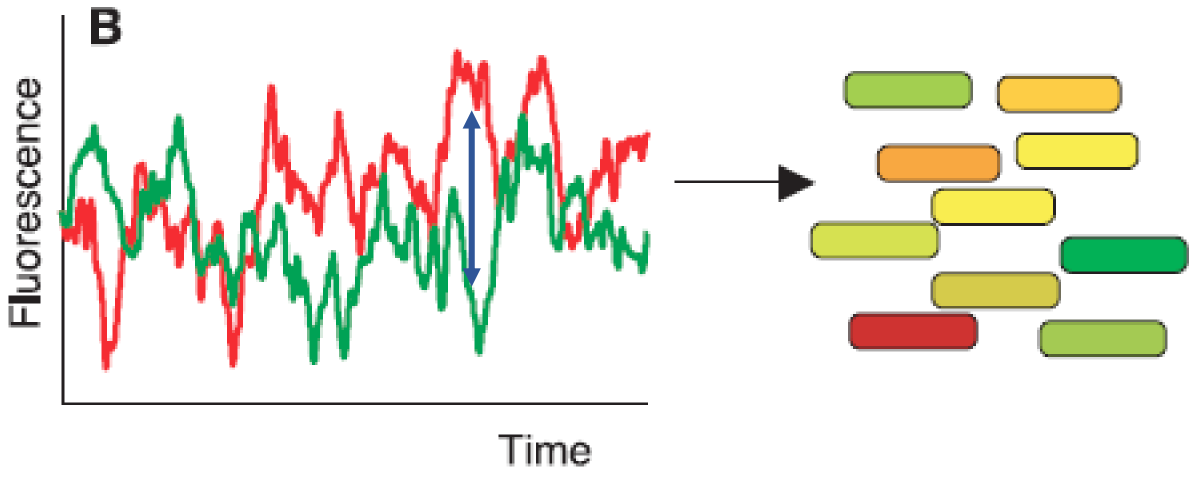


Heterogeneity between cells or of a cell over time

Surprisingly, with (quasi-identical) genetic parameters, expression from the same promoter is not the same within the cell at a given time.

=> Intrinsic noise

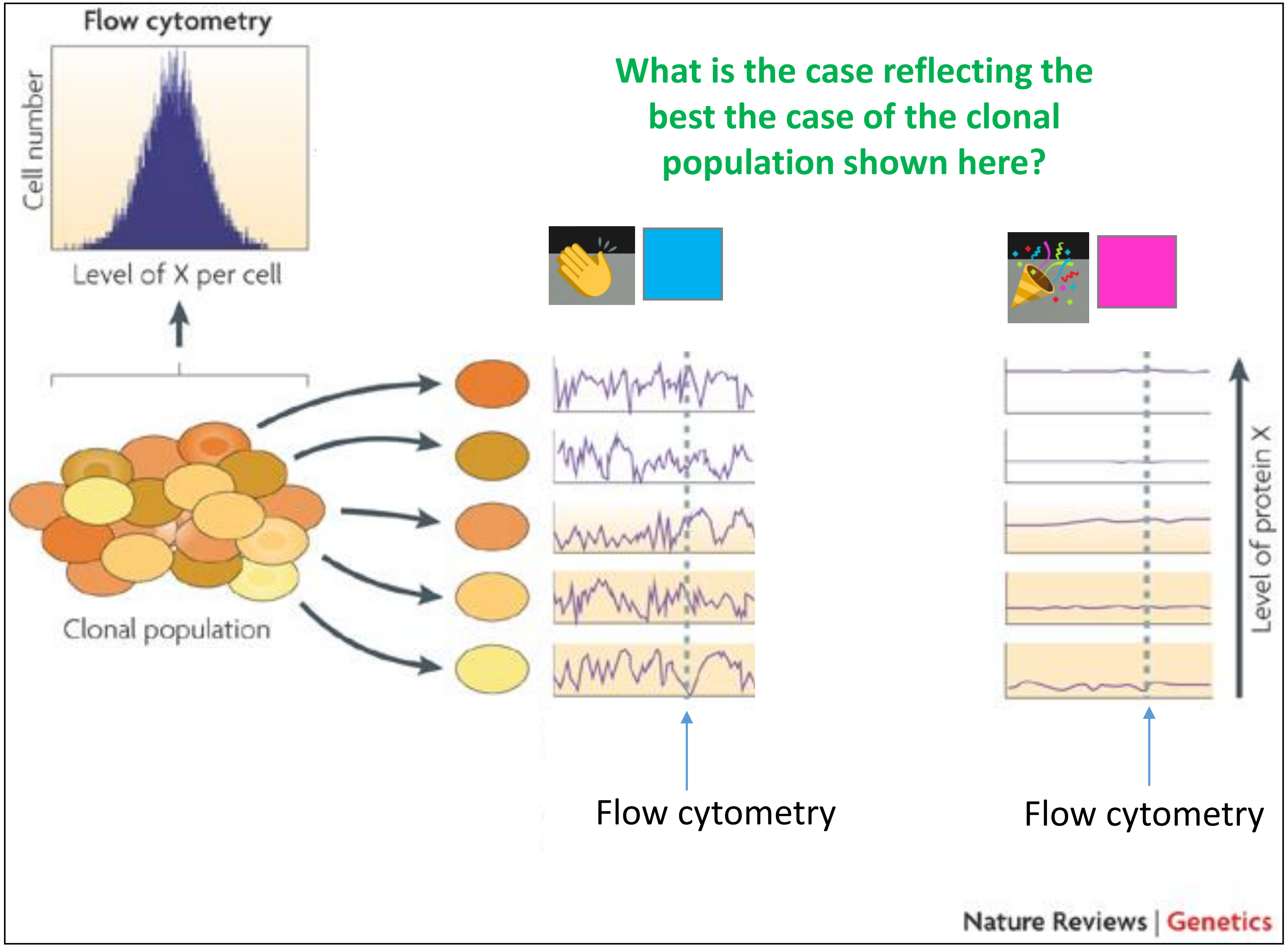
(stochasticity inherent in the biochemical process of gene expression)



Inherent noise in the gene expression process and intracellular heterogeneity

Elowitz *et al.*, *Science* (2002)

Noise components: temporal noise *versus* population



What is the case reflecting the best the case of the clonal population shown here?

👏 🟦

🎉 🟪

Flow cytometry

Flow cytometry

Zernicka-Goetz & Huang, *Nature Genetics Reviews* (2010)

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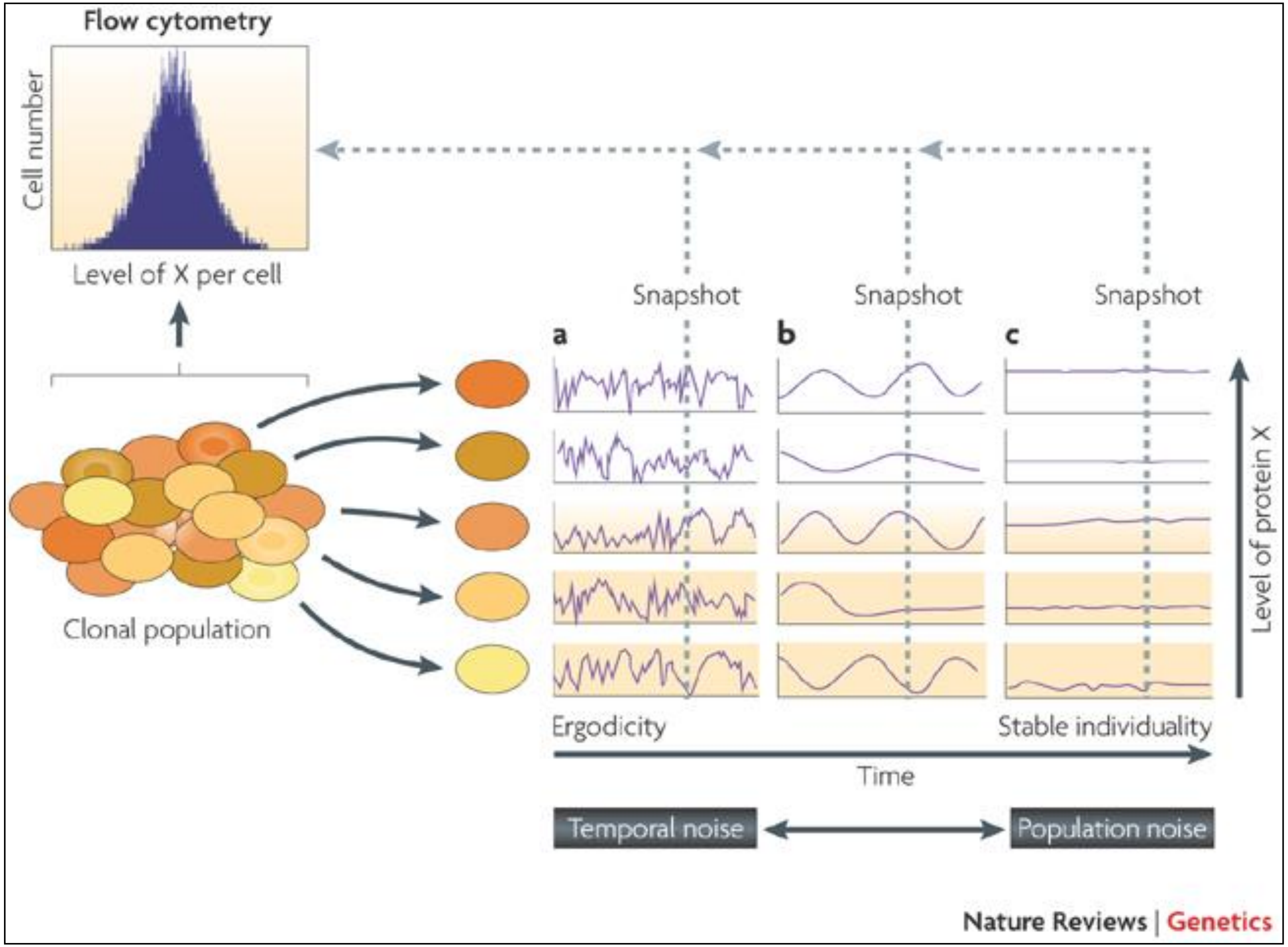
Noise components: temporal noise *versus* population



Zernicka-Goetz & Huang, *Nature Genetics Reviews* (2010)

Noise Components

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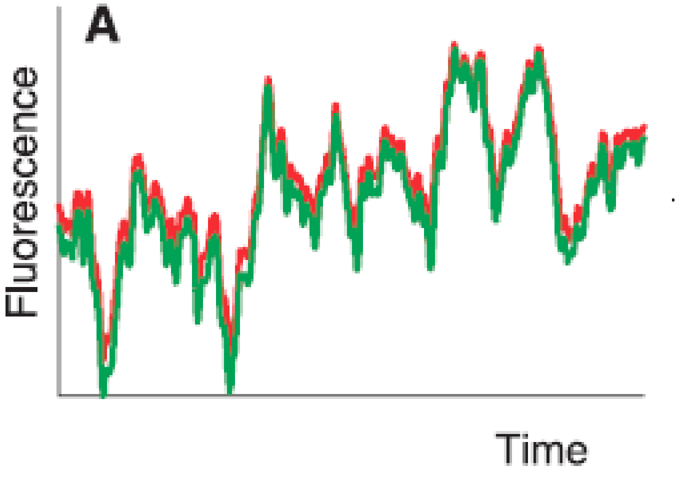
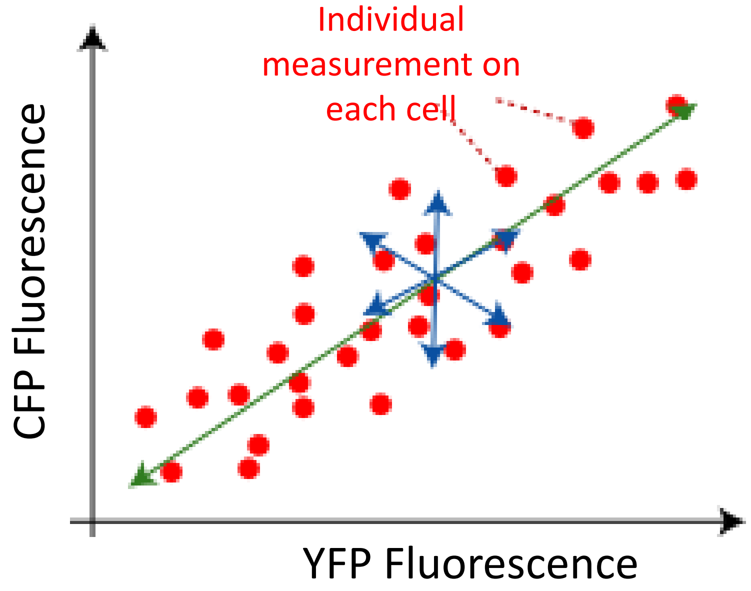
=> Extrinsic noise





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=> Intrinsic noise

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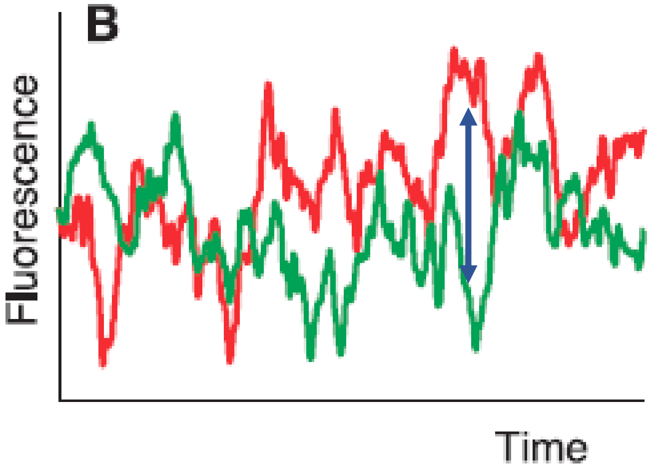

Which line represents extrinsic noise?

The blue or the green?

🟦 🟩

👏 🎉

Elowitz *et al., Science* (2002)

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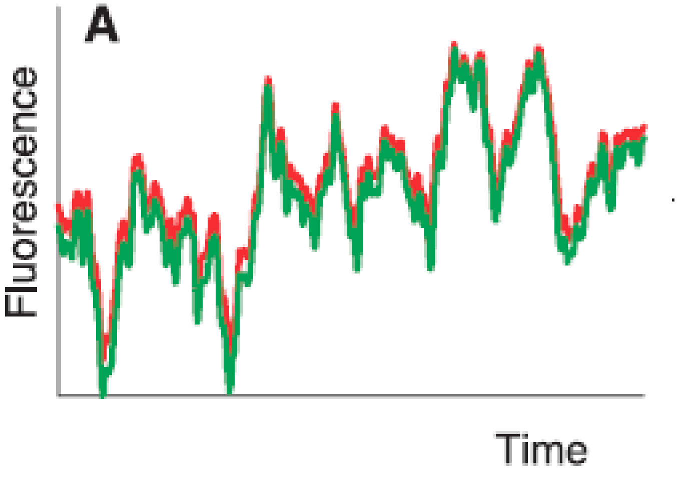
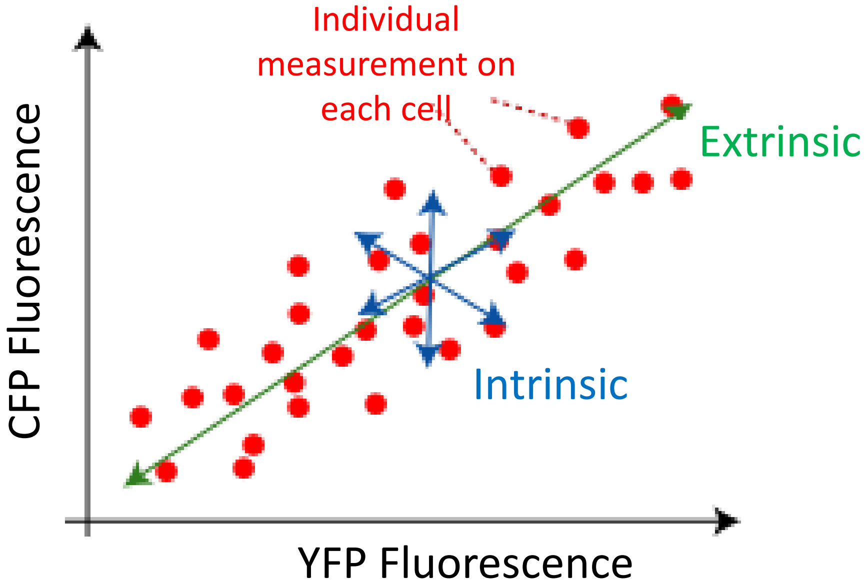
Noise Components

Variations in parameters modulating promoter activity and gene expression (post-transcriptional regulations) identically on both promoters.
Difference between cells or over time but not within the cell itself at a given time.

=> Extrinsic noise

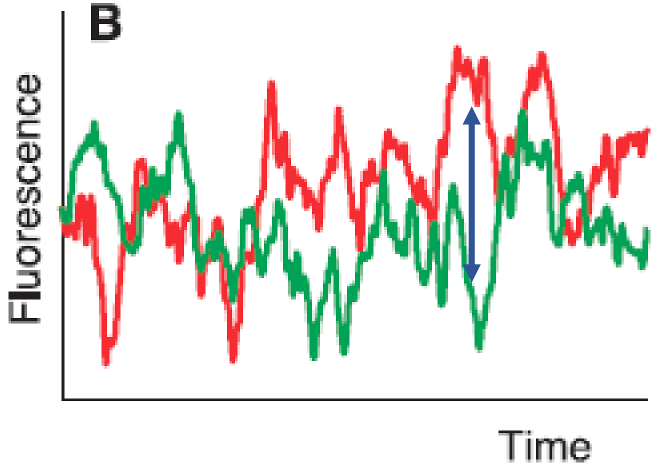




Surprisingly, with (quasi-identical) genetic parameters, expression from the same promoter is not the same within the cell at a given time.

=> Intrinsic noise

(stochasticity inherent in the biochemical process of gene expression)



Extrinsic noise ≈ covariance

Intrinsic noise ≈ deviation from the linear regression line

Total noise measured by the **coefficient of variation :** CV = $\frac{\sigma}{\mu}$

or the **Facteur Fano** : F = $\frac{\sigma^2}{\mu}$

$\mu = mean, \sigma^2 = variance, \sigma = standard\ deviation$

Elowitz *et al., Science* (2002)

High intrinsic noise, A or B ?

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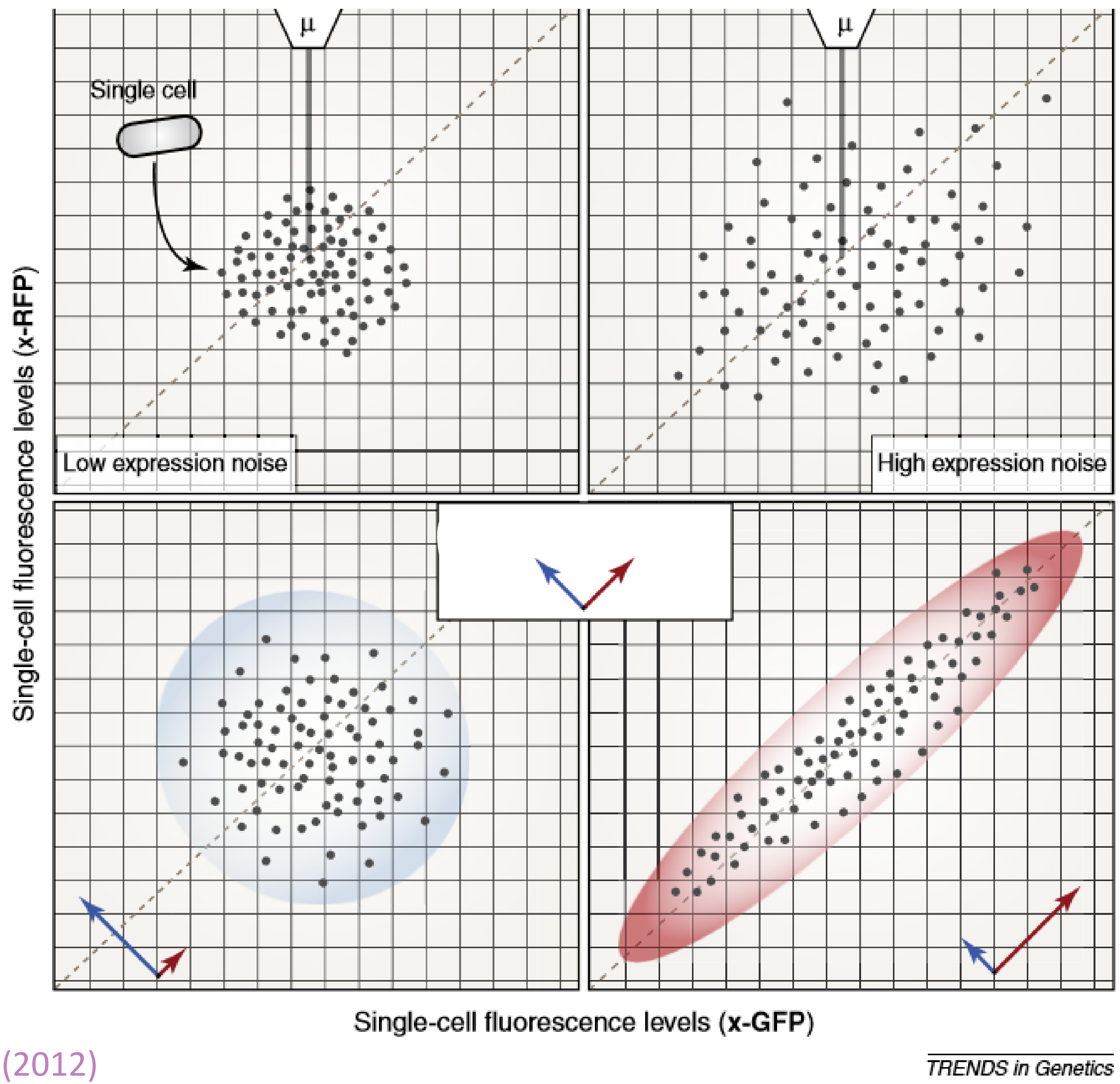

Chalancon *et al.*, *Trends in Genetics* (2012)

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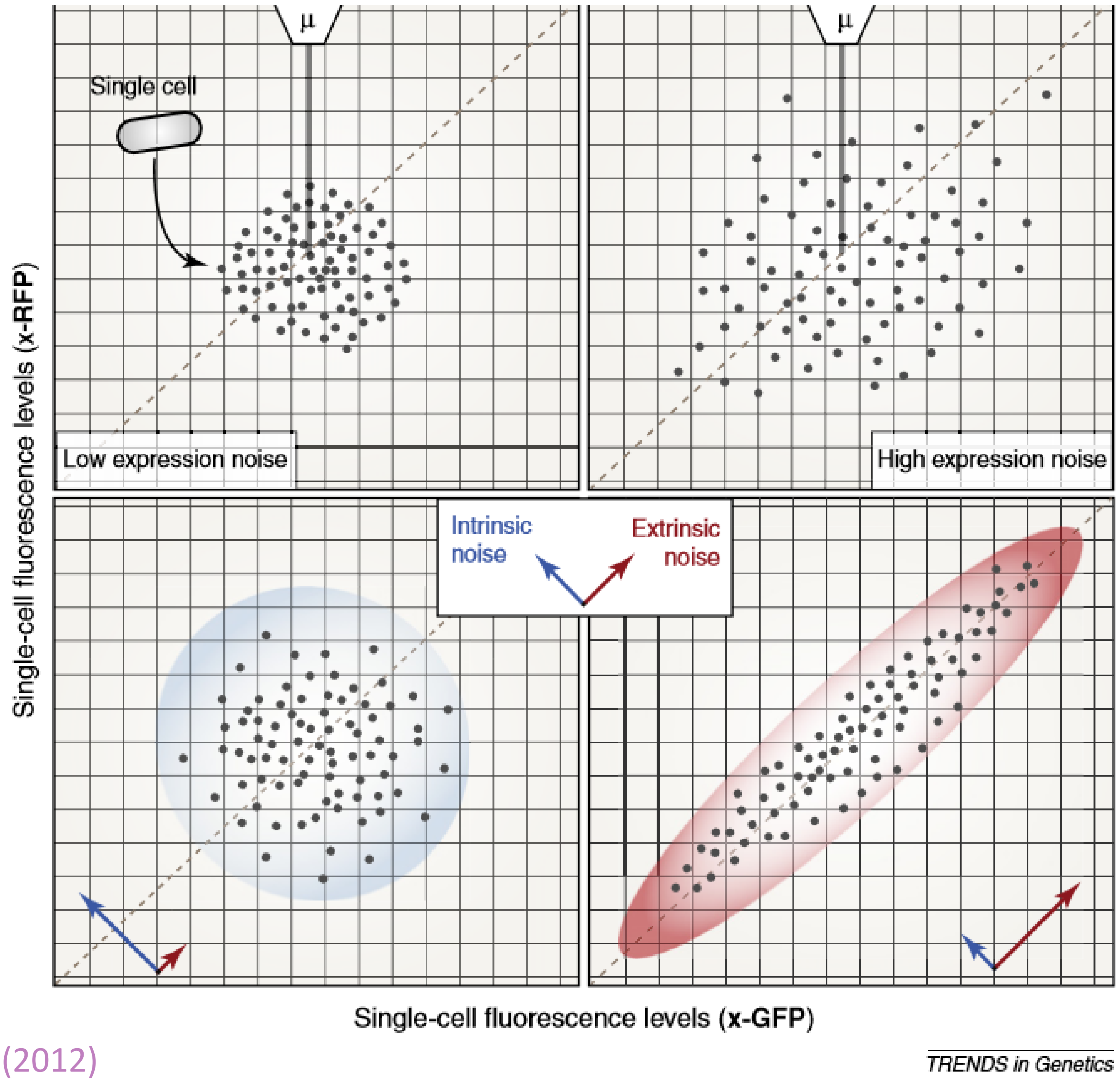


Chalancon *et al., Trends in Genetics* (2012)

Noise Components

Intrinsic noise *(difference in expression of a given promoter within the same cell)*

No intrinsic noise

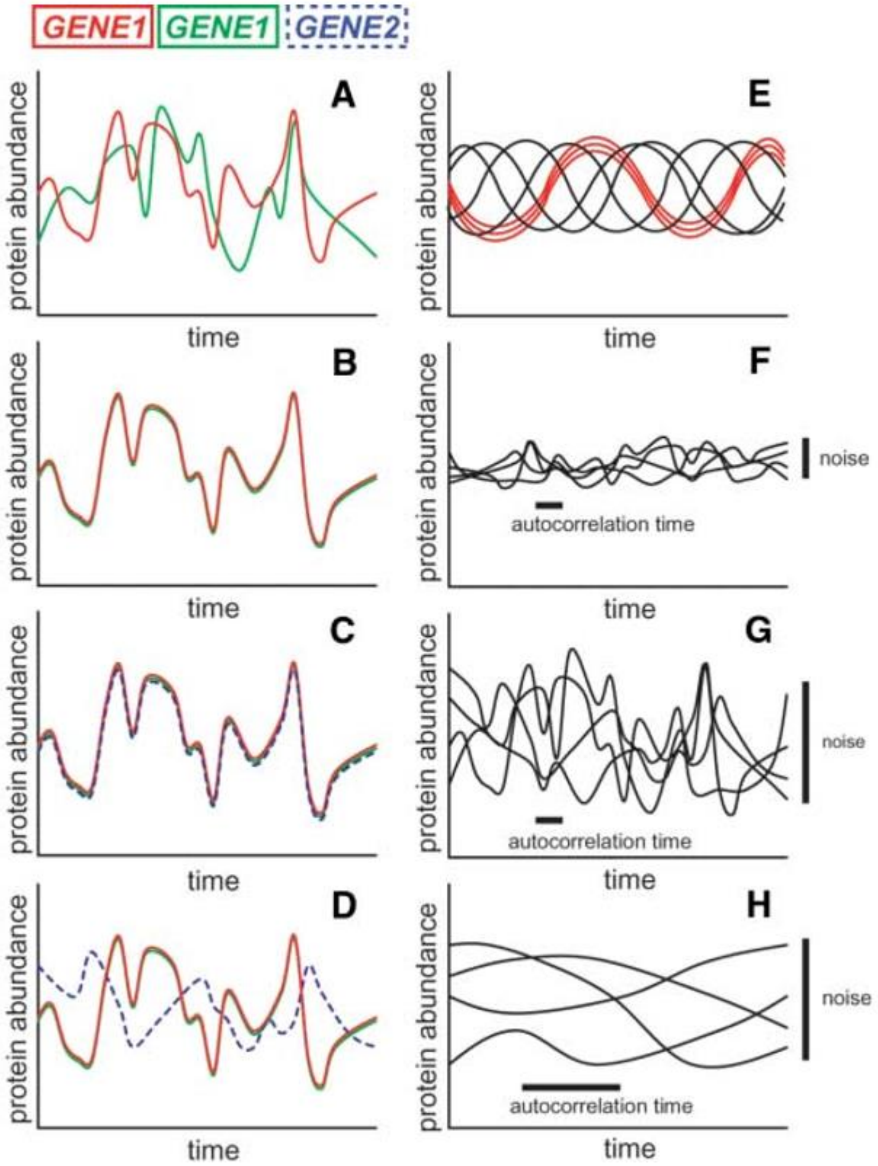
Extrinsic noise

+

Global noise
(fluctuations in the rate of basic reactions that affect the expression of all genes)

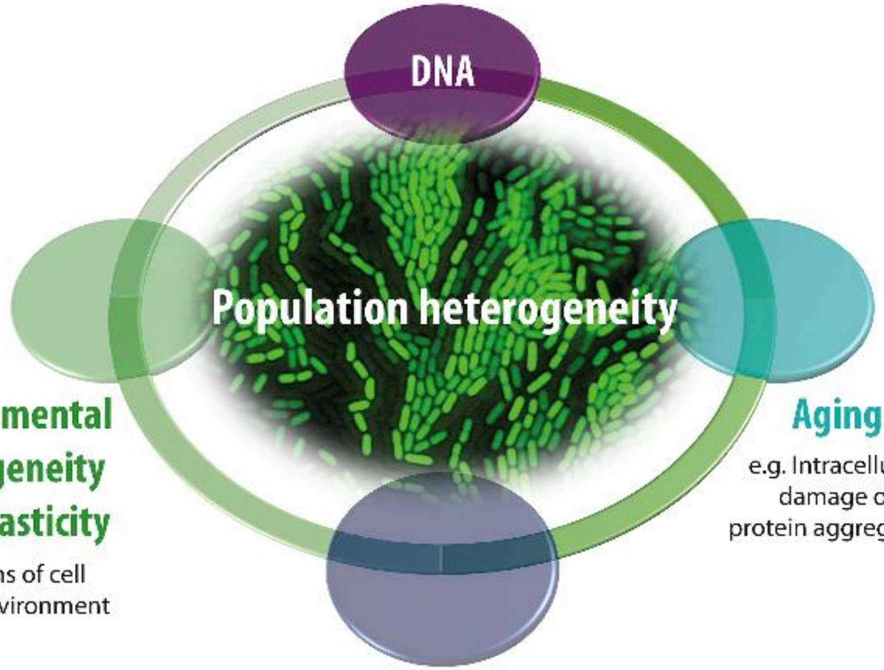
Specific extrinsic noise
(specifically affecting a given pathway/gene)

Within a cell **Within the population**



Each black line represents a given cell.
The red lines represent a synchronization case

Raser & O'Shea, *Science* (2005)



Genetic variation

e.g. Mutations,
Phase variation

Environmental heterogeneity & stochasticity

Variations of cell
(micro-)environment

Aging

e.g. Intracellular
damage or
protein aggregation

Stochasticity of gene expression

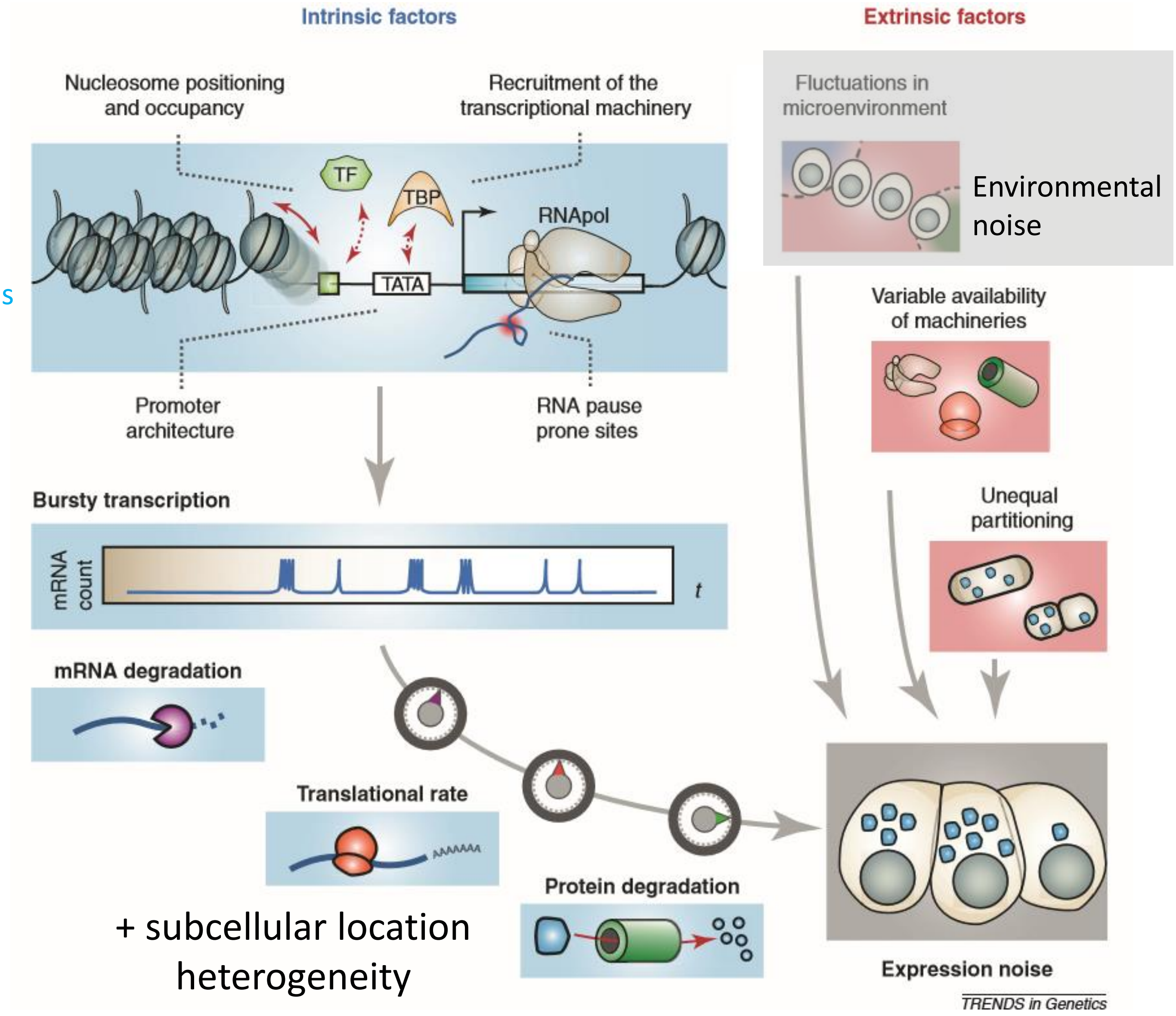
Fluctuations in the set of reactions
that control the abundance of gene products

- **Intrinsic**: Differences in gene expression between identical gene copies in a single cell
- **Extrinsic**: Differences from cell to cell or in a single cell over time that affect all the copies of the same gene equally
  - **Global**: Differences that affect almost all genes
  - **Specific**: Differences that affect specifically TF- and signaling pathway- regulated genes

Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

The origin of noise?

For instance in Eukaryotes
(similar in Prokaryotes)



Environmental noise

+ subcellular location heterogeneity

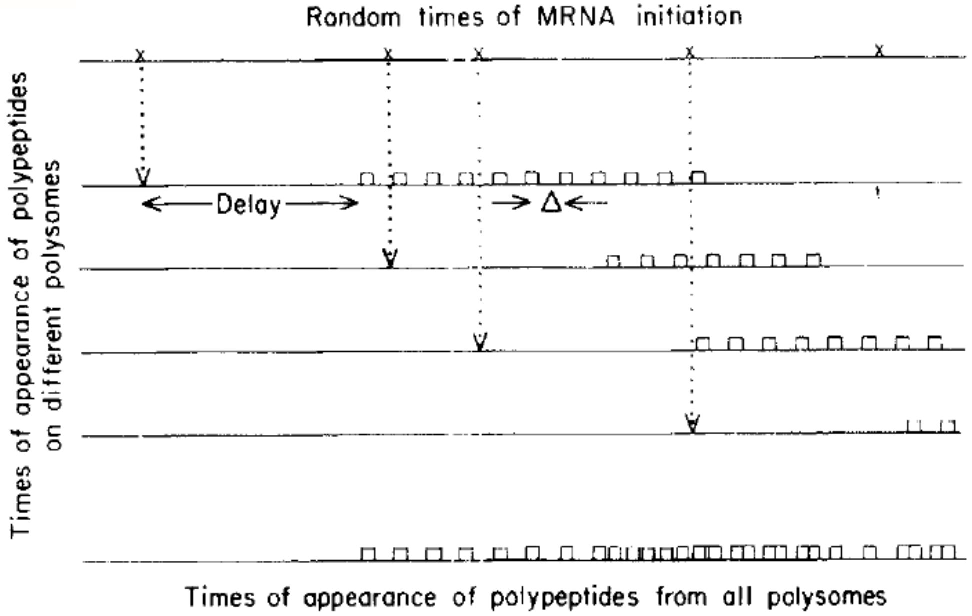
Origins :
- Thermal noise
- Biochemical noise
- **Effect of small number**
- Intracellular gradients
- Quantum Noise (*stochastic fluctuations in the behavior of molecules on atomic scales*)

One often distinguishes:
- **Molecular causes: Process efficiency ≠ 100%**
- **Low regulator concentration**
- Topological causes - structure of chromatin

See Bionumbers
http://bionumbers.hms.harvard.edu/

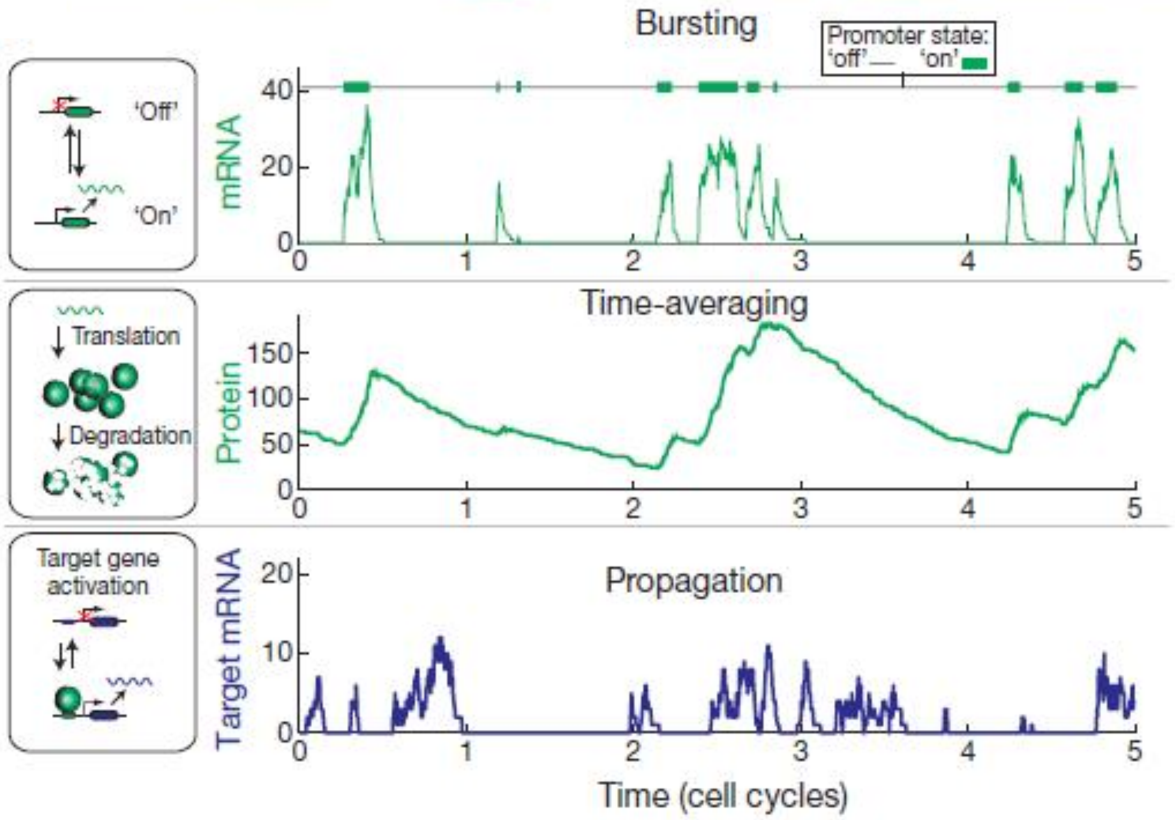
Chalancon *et al., Trends in Genetics* (2012)

Life is bursts



Model of polysome kinetics. At random times, different RNA polymerase molecules initiate synthesis of mRNA at the promoter of a gene (top line). Following a delay, δ, completed polypeptide chains will be produced from each messenger; a time interval Δ separates the times of appearance of successive polypeptides on a given messenger; the number of polypeptides per messenger is random (middle four lines). The times of appearance of polypeptides correspond to the superposition of the times of appearance on all of the polysomes (bottom line). The random variable N_t (number of polypeptides in the cell at time t) is found by counting the number of polypeptide appearances to the left of the position on the bottom line corresponding to time t.

Rigney, *J. theor. Biol.* (1979)



Mechanisms that shape noise in gene expression. Noise is characterized by bursty expression of mRNA (top). Proteins typically have longer lifetimes than bursts, leading them to time-average or 'buffer' these bursts (middle). Finally, noise in one gene can propagate to generate further noise in the expression of downstream genes (bottom).

Elowitz *et al.*, *Nature* (2010)

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A stochastic cell model linking gene expression, metabolism & replication to predict growth

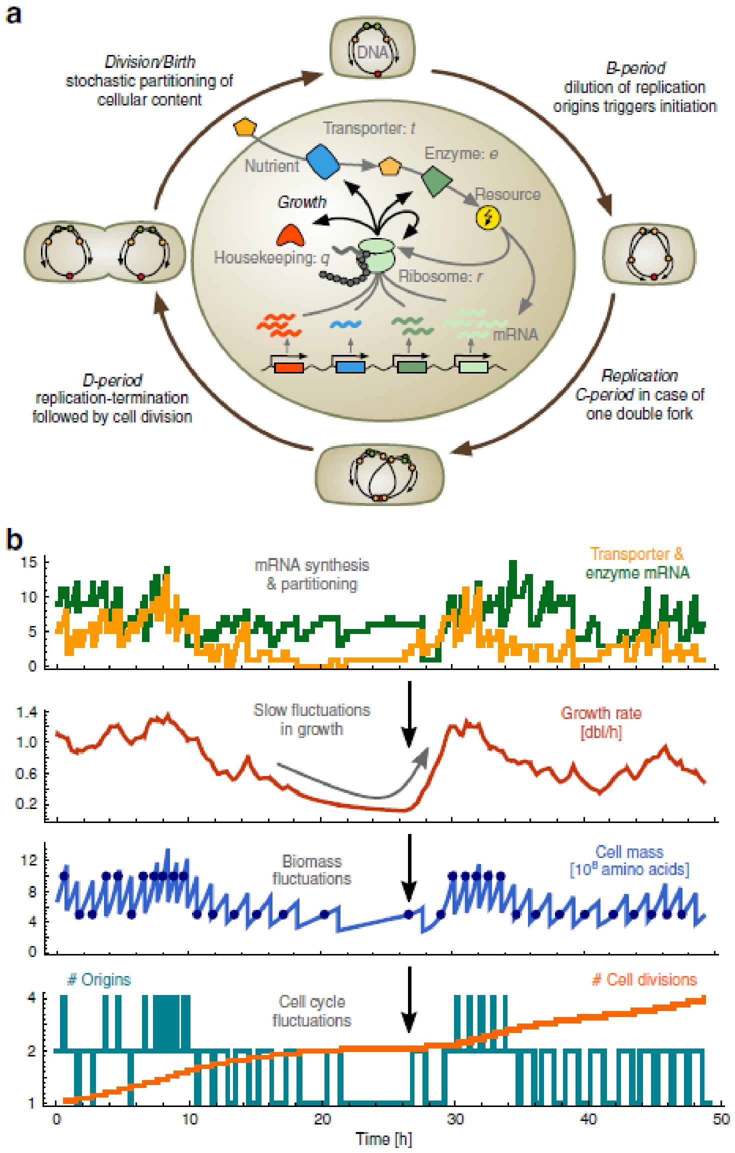

Fig. 1 Stochastic model of single-cell growth. **a** The outer cycle illustrates the cell cycle model based on the Cooper-Helmstetter model of bacterial replication. We assume initiation of a new round of replication at a fixed concentration of DNA-origins, analogous to a fixed initiation mass per DNA-origin[25], thus growth dynamics schedule the replication events and are determined by the intracellular model (inner circle). The latter describes import and metabolism of resources, and how they fuel gene expression, where the rate of protein-biosynthesis determines growth. Stochasticity of cellular dynamics is a result of the intrinsic stochasticity of the various reactions and the random partitioning of the cellular content at division. **b** Stochastic simulations illustrate the propagation of intrinsic fluctuations in single cells: mRNAs are synthesised at low numbers per cell (yellow & green lines), which affects protein production and so growth rate (red line). Fluctuations in growth lead to temporal variations in cell mass that can span several cell cycles (blue line), causing fluctuations in the number of replication origins (teal line), in the mass at initiation (filled circles), and consequently in cell divisions (orange line)

What the model tells us:

- Transcription & cell partitioning at division are the major determinant of growth heterogeneity in most conditions.
- Synthesis and removal of mRNAs encoding nutrient transporters have a major contribution to growth variation.

Thomas *et al., Nature Communications* (2018)

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A stochastic cell model linking gene expression, metabolism & replication to predict growth

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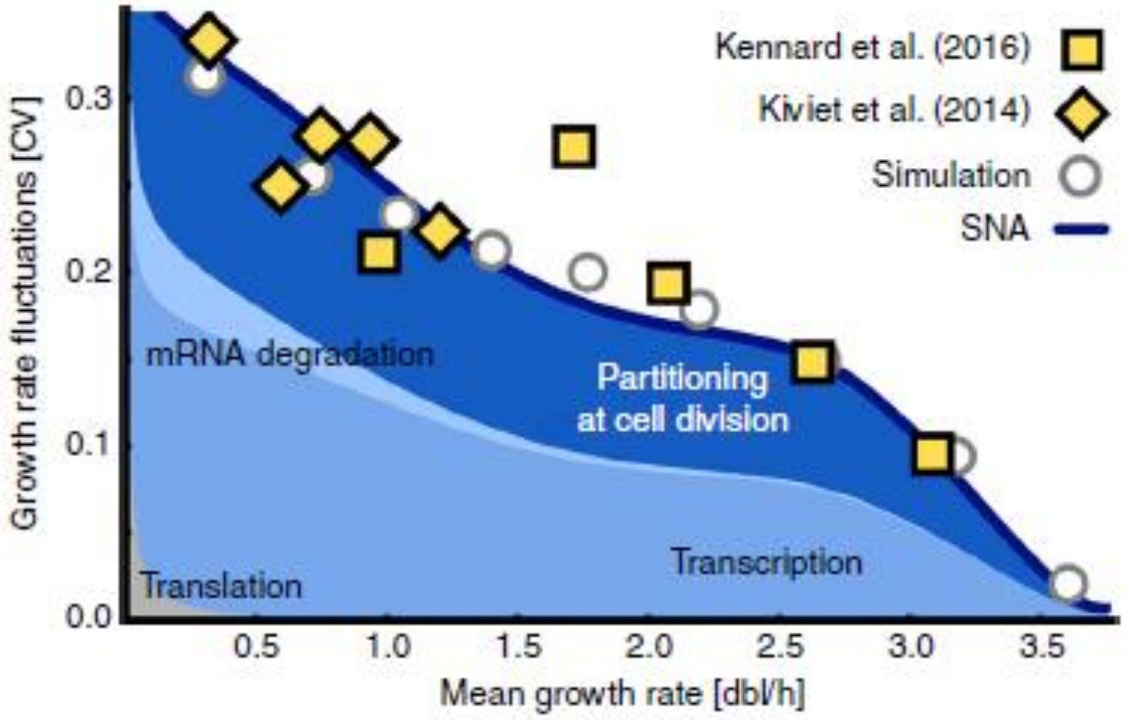
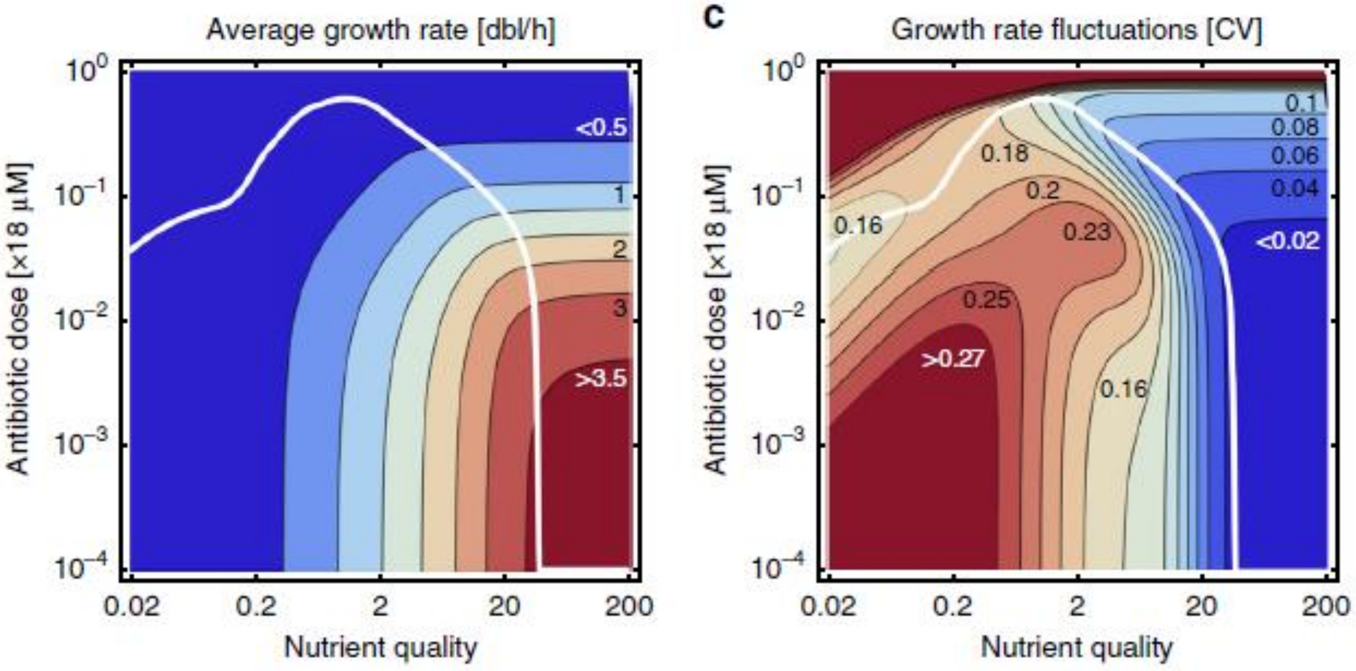


Thomas *et al.*, *Nature Communications* (2018)

A stochastic cell model linking gene expression, metabolism & replication to predict growth

What the model tells us:

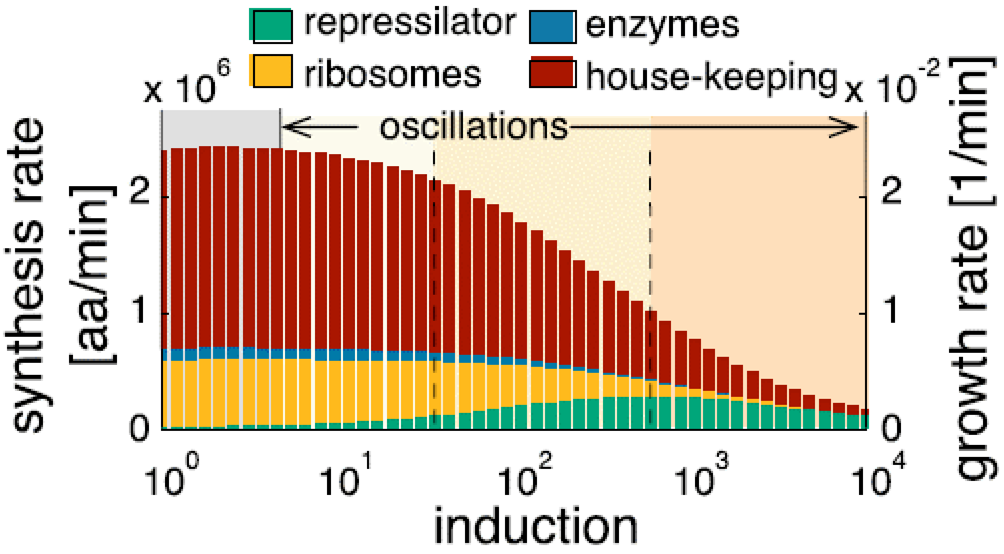
- Transcription & cell partitioning at division are the major determinant of growth heterogeneity in most conditions.
- Synthesis and removal of mRNAs encoding nutrient transporters have a major contribution to growth variation.
- Environmental conditions can have an impact on growth variation. Especially, growth heterogeneity exhibits a complex pattern in presence of antibiotics => possible impact on tolerance to antibiotics.

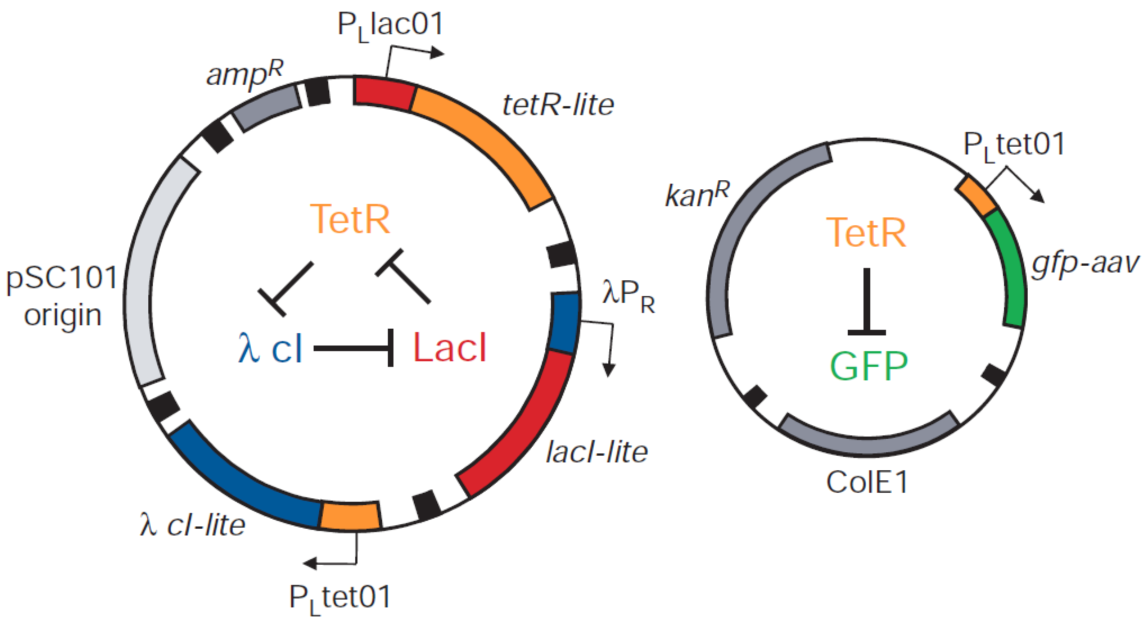


White line = non-zero drug dose that minimises growth heterogeneity

Thomas *et al.*, *Nature Communications* (2018)

Modelling the impact of a synthetic circuit?





How does the repressilator behaves?

(Find an analogy)

Previous deterministic model of cell growth
Weibe *et al., PNAS* (2015)

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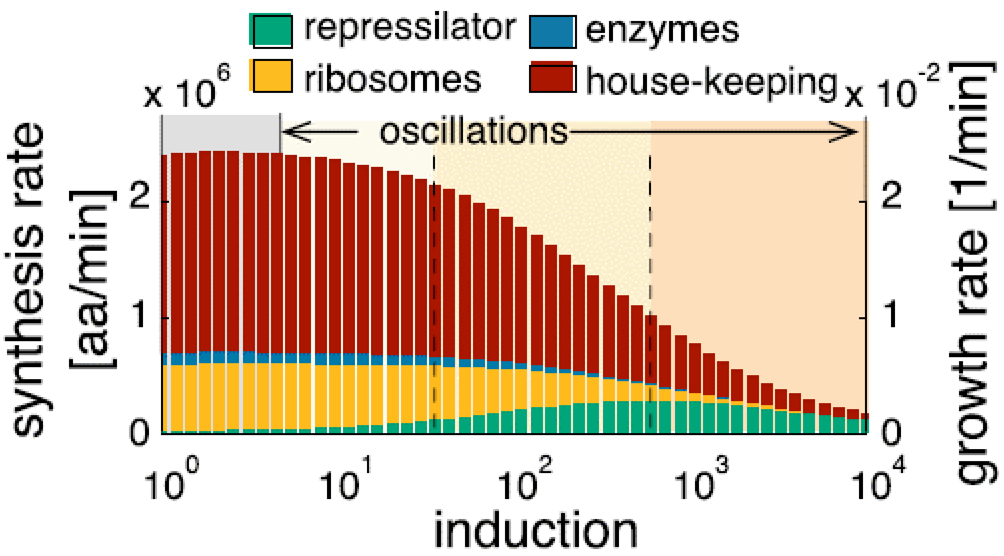
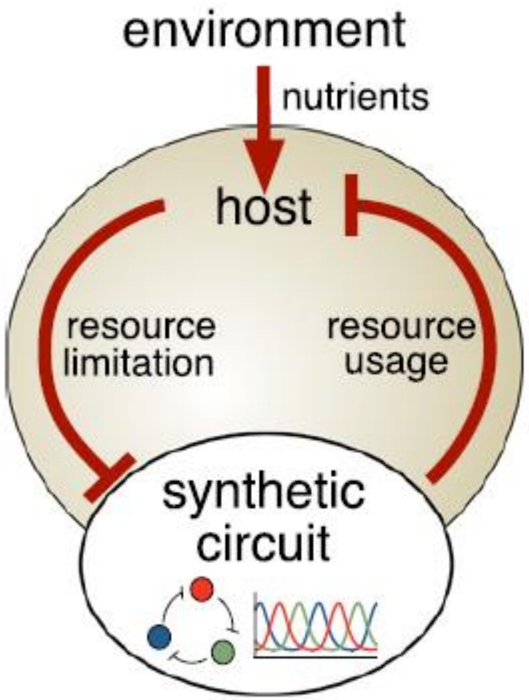
Modelling the impact of a synthetic circuit?



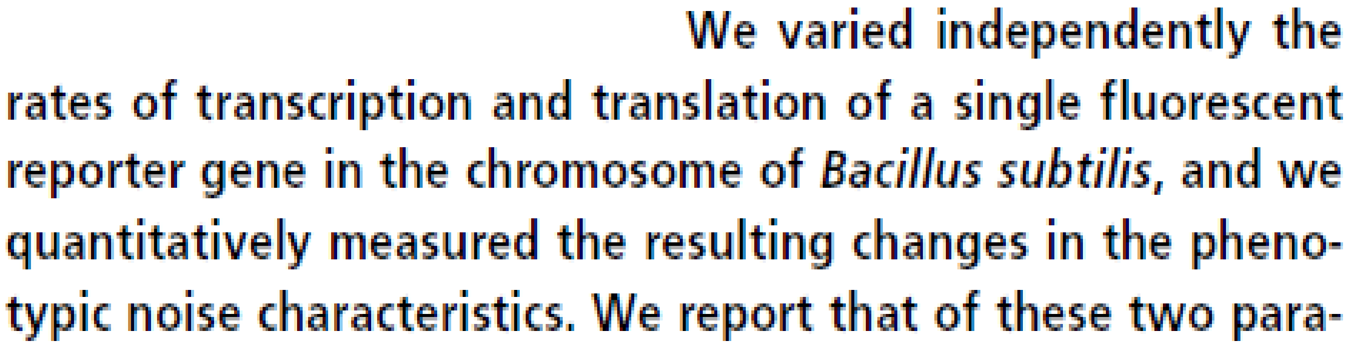


Like a molecular parasite

Previous deterministic model of cell growth
Weibe *et al., PNAS* (2015)

Can the noise be genetically encoded?

Cause of noise in the expression of prokaryotes: transcription *versus* translation?


We varied independently the rates of transcription and translation of a single fluorescent reporter gene in the chromosome of *Bacillus subtilis*, and we quantitatively measured the resulting changes in the phenotypic noise characteristics. We report that of these two para-

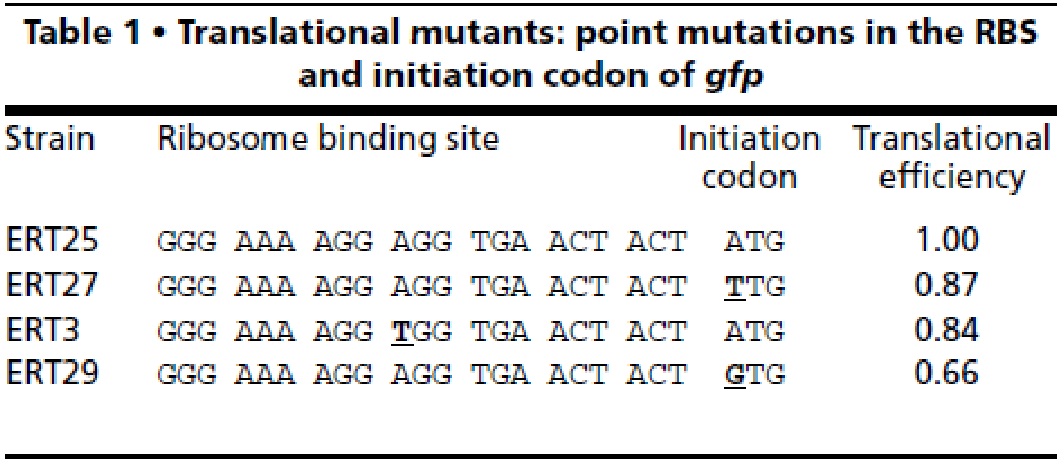


Table 1 • Translational mutants: point mutations in the RBS and initiation codon of *gfp*

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	**T**TG	0.87
ERT3	GGG AAA AGG **T**GG TGA ACT ACT	ATG	0.84
ERT29	GGG AAA AGG AGG TGA ACT ACT	**G**TG	0.66

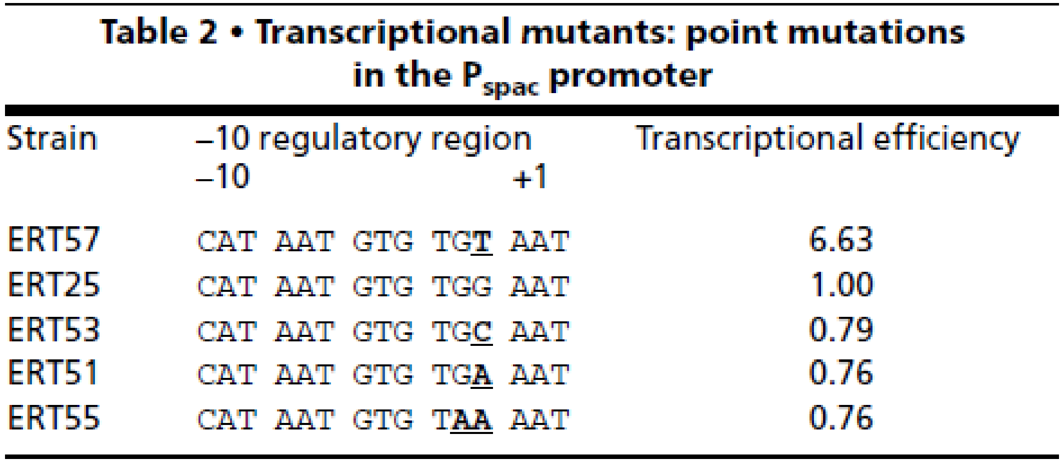


Table 2 • Transcriptional mutants: point mutations in the P_{spac} promoter

Strain	–10 regulatory region –10 +1	Transcriptional efficiency
ERT57	CAT AAT GTG TG**T** AAT	6.63
ERT25	CAT AAT GTG TGG AAT	1.00
ERT53	CAT AAT GTG TG**C** AAT	0.79
ERT51	CAT AAT GTG TG**A** AAT	0.76
ERT55	CAT AAT GTG T**AA** AAT	0.76

Ozbudak *et al.*, *Nature Genetics* (2002)

Highest cause of noise in the expression : transcription or translation?

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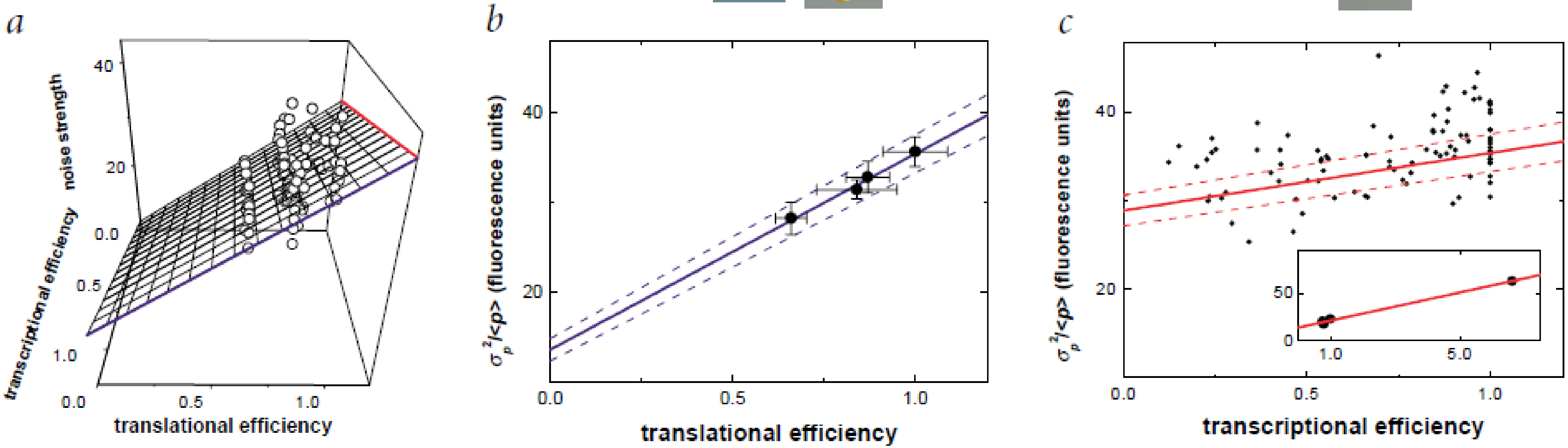
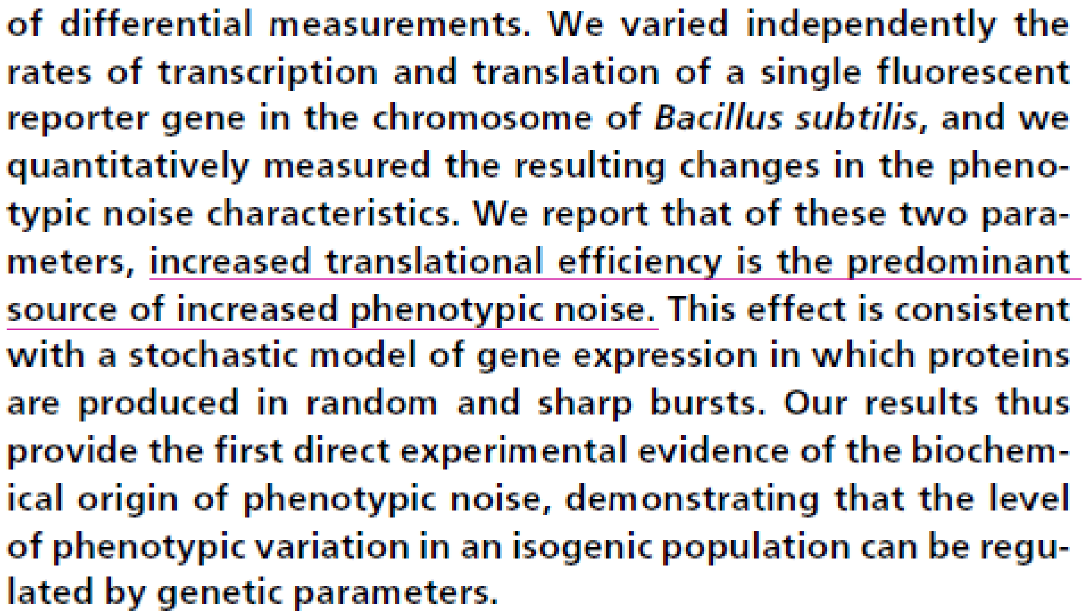


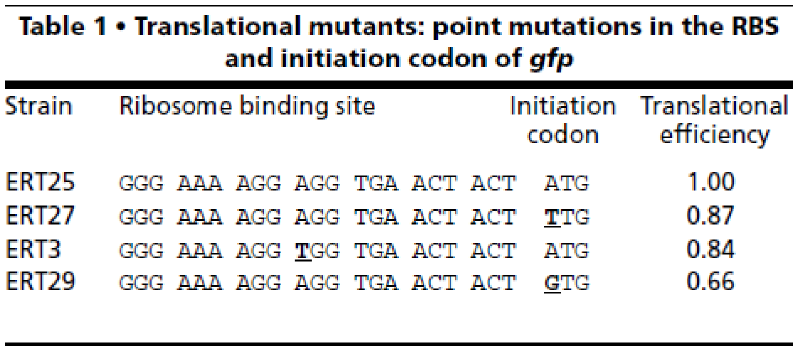
Fig. 2 Biochemical contribution to phenotypic noise. *a*, Complete experimental data. Each data point is the summarized result of an entire histogram corresponding to a flow cytometer run of a population of typically 10^4–10^5 cells. The phenotypic noise strength of the population (*z*, in arbitrary fluorescence units) is plotted as a function of transcriptional efficiency (*x*, depending on the IPTG concentration) and translational efficiency (*y*, depending on the translational mutant used). Transcriptional and translational efficiencies are normalized to those of the wildtype ERT25 strain, allowing these parameters to be directly compared. These data are fitted to a plane of the form $z=a_0+a_x x+a_y y$ using a least-square routine, giving $a_0=7.1 \pm 0.9$, $a_x=6.5 \pm 0.4$, $a_y=21.8 \pm 0.9$. The ratio $a_y/a_x=3.4$ gives the relative effect of translational versus transcriptional efficiency on phenotypic noise strength. *b*,*c*, For clarity, the three-dimensional data are projected parallel to the fit plane onto the boundary planes $x=1$ (*b*), noise strength as a function of translation, and $y=1$ (*c*), noise strength as a function of transcription. The intersection of the fit plane with each boundary plane is shown as a solid line; dotted lines indicate an interval of 1 s.d. Data in *b* are summarized separately for each translational mutant (dark circles with error bars that represent 95% c.i.). Inset in *c* shows results of control experiments conducted on transcriptional mutants at full induction. Three strains (ERT51, ERT53 and ERT55) are very similar, both in transcriptional efficiency and in noise strength, suggesting that biochemical noise is determined by the actual transcription rate rather than by the specific method used to achieve it. The strain ERT57 shows a highly amplified transcriptional efficiency, allowing reliable estimation of correlations. Data are summarized separately for each transcriptional mutant. A linear fit through these points gives a slope $a_x'=7.3 \pm 0.3$, which is consistent with the slope $a_x=6.5 \pm 0.4$ obtained from *a*.

Ozbudak *et al.*, *Nature Genetics* (2002)

Cause of noise in the expression of prokaryotes: transcription versus translation?



of differential measurements. We varied independently the rates of transcription and translation of a single fluorescent reporter gene in the chromosome of *Bacillus subtilis*, and we quantitatively measured the resulting changes in the phenotypic noise characteristics. We report that of these two parameters, increased translational efficiency is the predominant source of increased phenotypic noise. This effect is consistent with a stochastic model of gene expression in which proteins are produced in random and sharp bursts. Our results thus provide the first direct experimental evidence of the biochemical origin of phenotypic noise, demonstrating that the level of phenotypic variation in an isogenic population can be regulated by genetic parameters.



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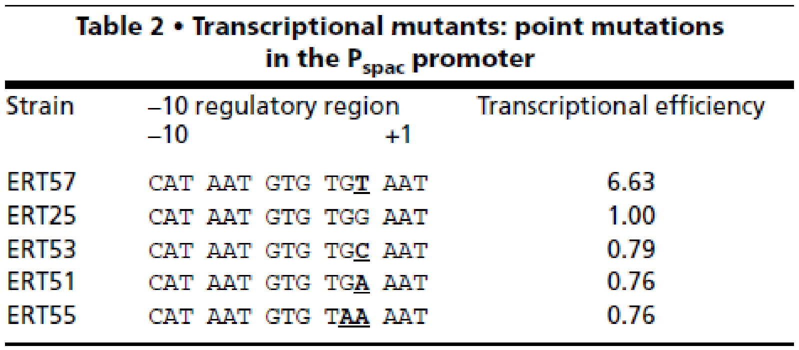

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Ozbudak *et al.*, *Nature Genetics* (2002)

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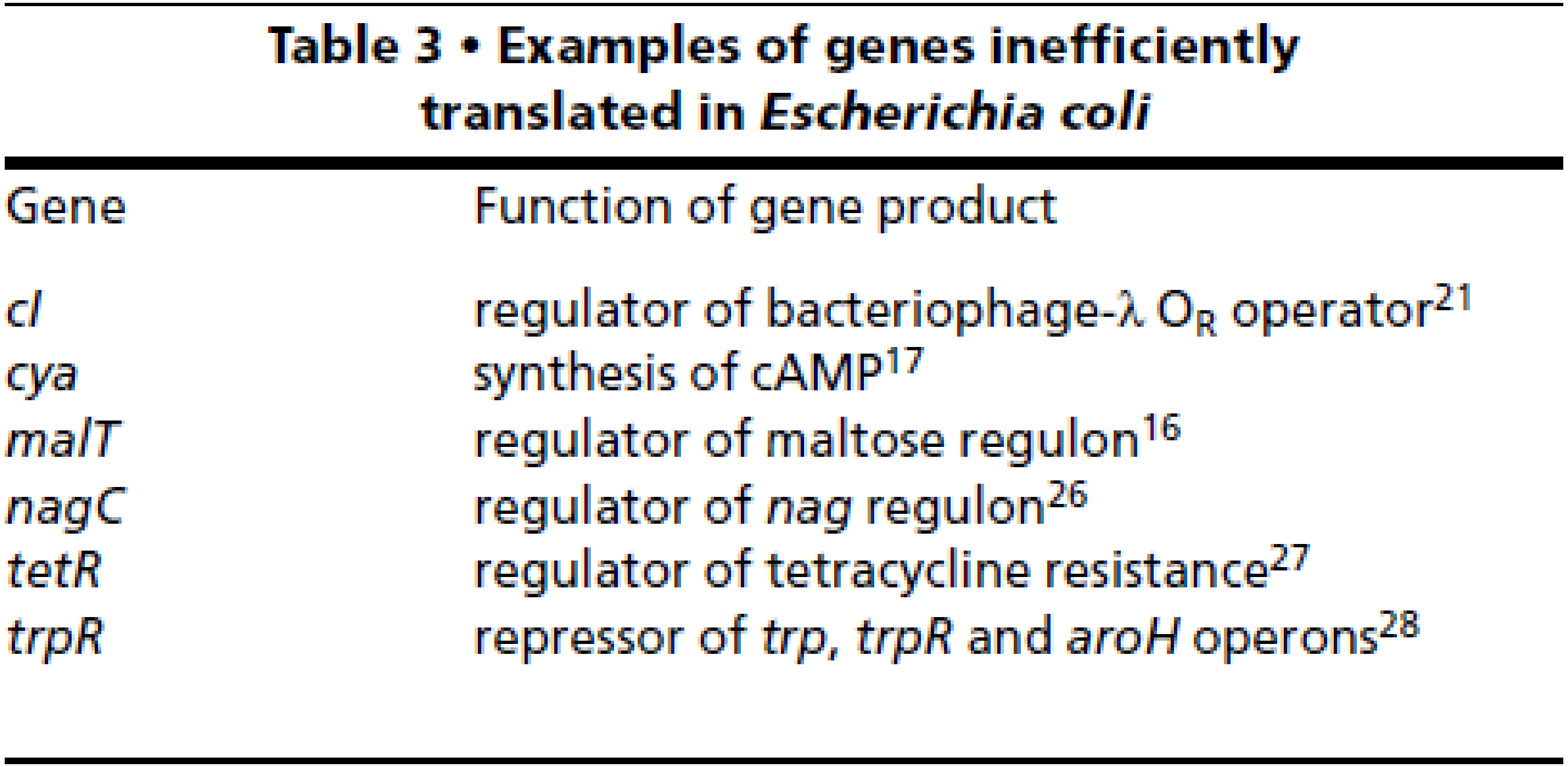

Table 3 • Examples of genes inefficiently translated in *Escherichia coli*

Gene	Function of gene product
cI	regulator of bacteriophage-λ O_R operator[21]
cya	synthesis of cAMP[17]
malT	regulator of maltose regulon[16]
nagC	regulator of *nag* regulon[26]
tetR	regulator of tetracycline resistance[27]
trpR	repressor of *trp*, *trpR* and *aroH* operons[28]

Ozbudak *et al.*, *Nature Genetics* (2002)

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Let’s play to the « noise game »

High or low level of noise?

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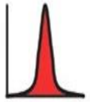

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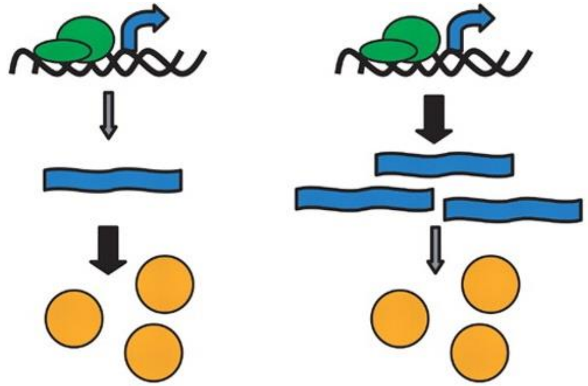
①

Infrequent transcription + efficient translation

②

Frequent transcription + inefficient translation

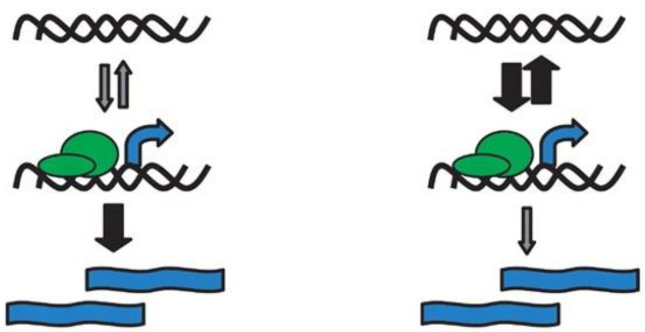


③

Infrequent promoter transitions between inactive and active states + efficient transcription

④

Frequent promoter transitions + inefficient transcription

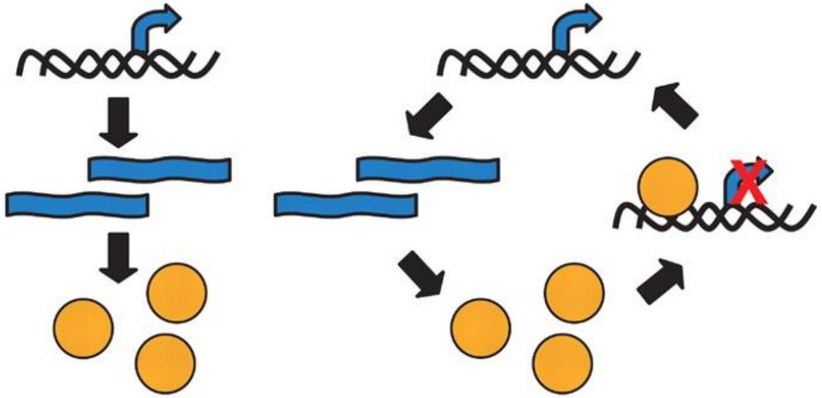


⑤

No negative feedback

⑥

Negative feedback



Raser & O’Shea, *Science* (2005)

Let’s play to the « noise game »

High or low level of noise?

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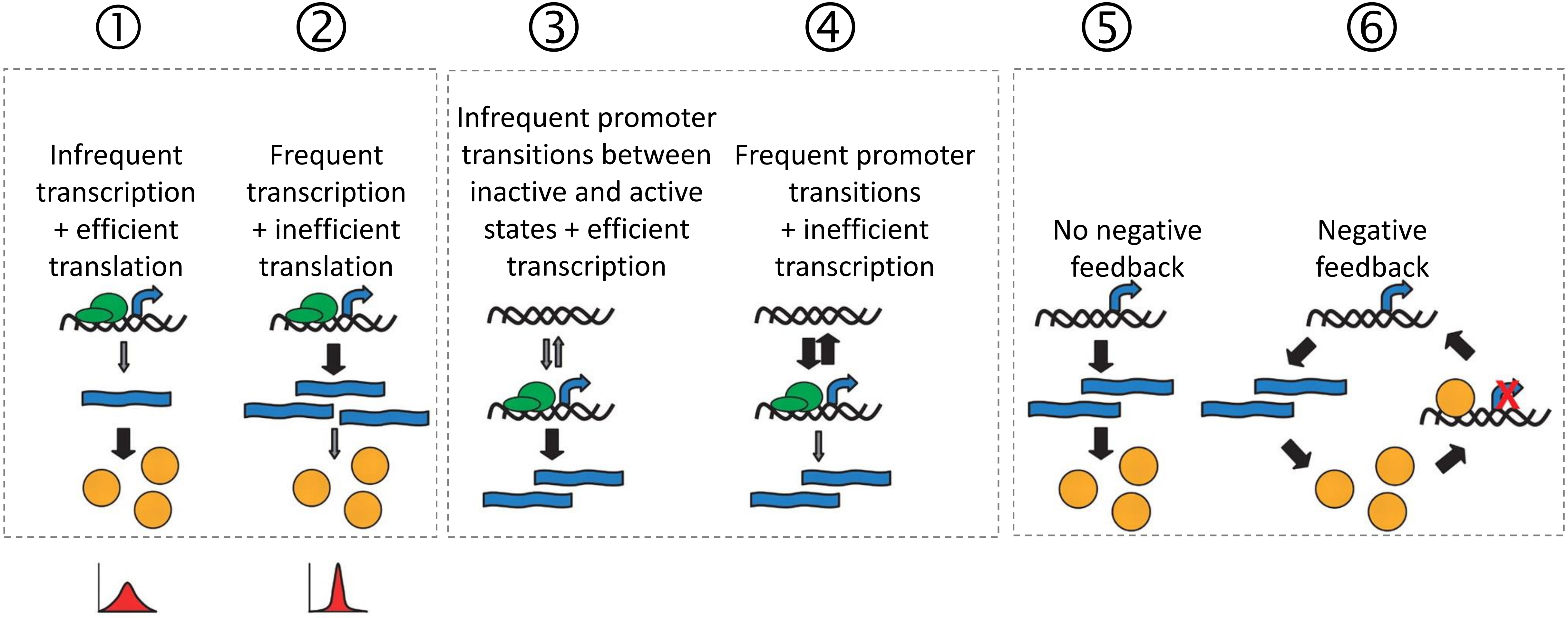


① Infrequent transcription + efficient translation

② Frequent transcription + inefficient translation

③ Infrequent promoter transitions between inactive and active states + efficient transcription

④ Frequent promoter transitions + inefficient transcription

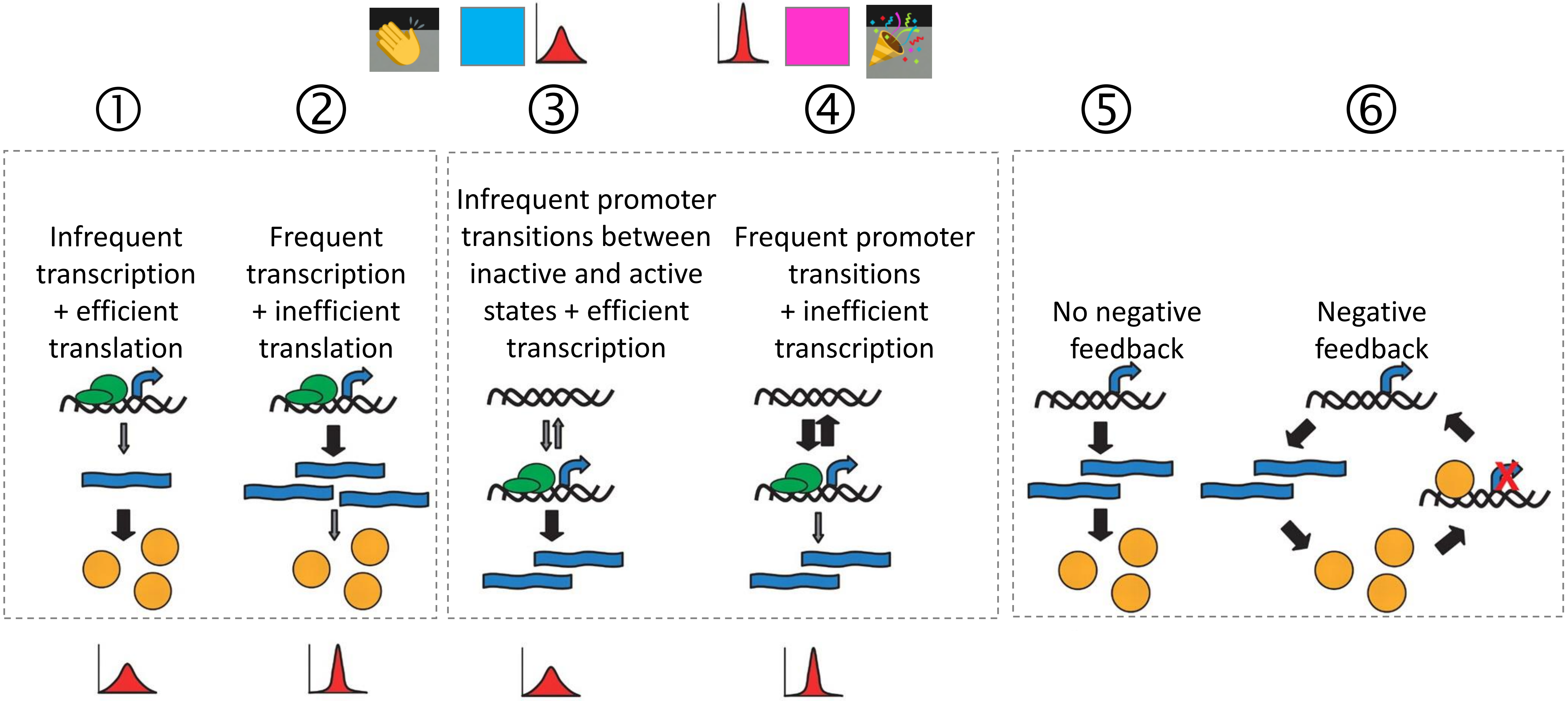
⑤ No negative feedback

⑥ Negative feedback

Raser & O’Shea, *Science* (2005)

Let’s play to the « noise game »

High or low level of noise?



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① Infrequent transcription + efficient translation

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③ Infrequent promoter transitions between inactive and active states + efficient transcription

④ Frequent promoter transitions + inefficient transcription

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⑥ Negative feedback

Raser & O’Shea, *Science* (2005)

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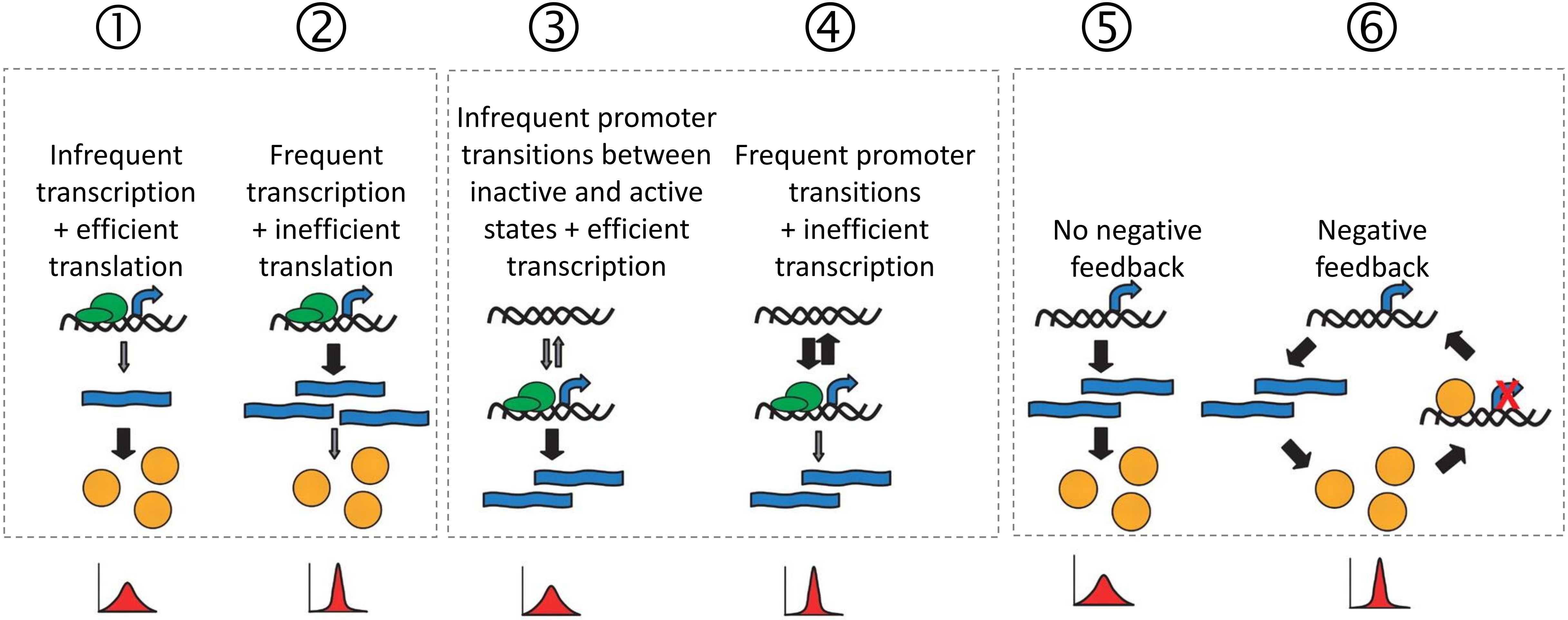
Let’s play to the « noise game »

High or low level of noise?



①
Infrequent transcription + efficient translation

②
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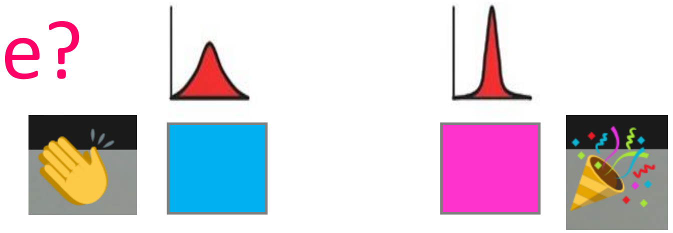
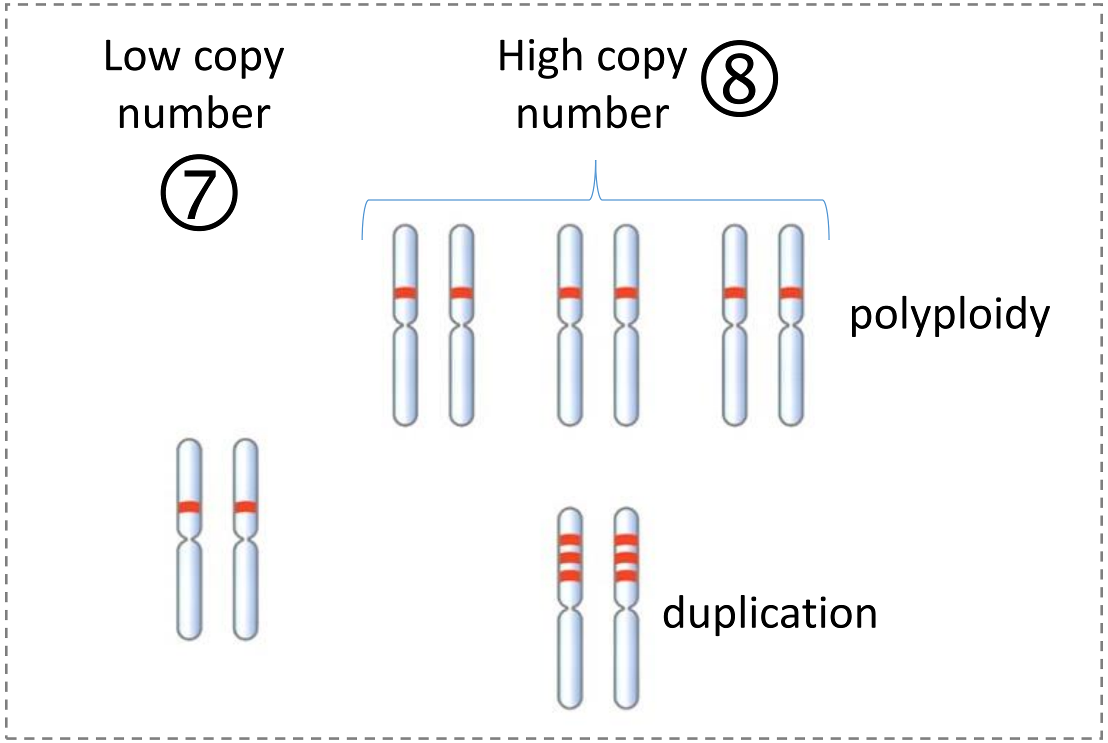
Raser & O’Shea, *Science* (2005)

Let’s play to the « noise game »

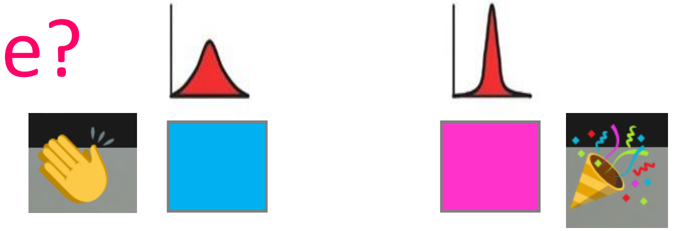
High or low level of noise?

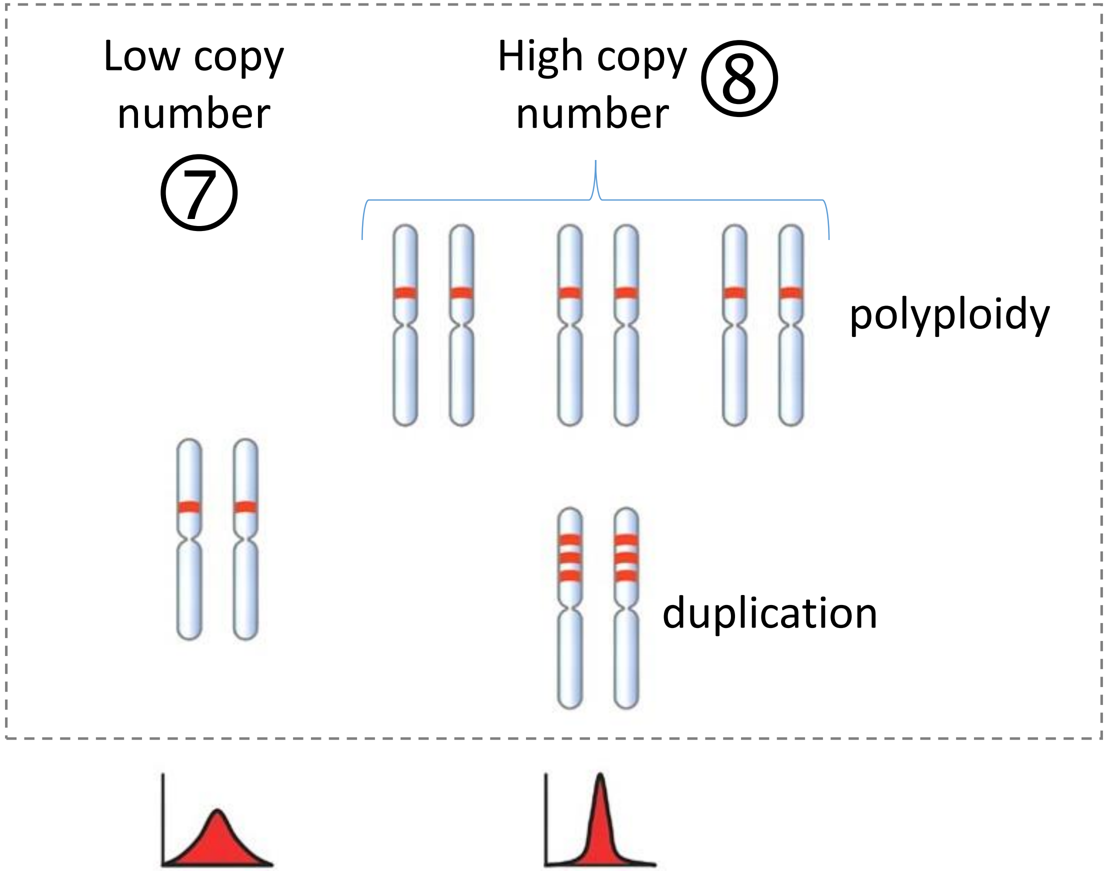




Let’s play to the « noise game »

High or low level of noise?

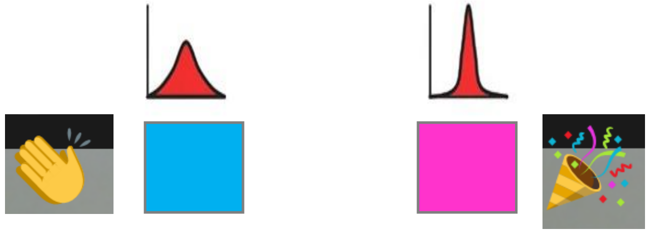




Noise and gene networks

Noise is "controllable"

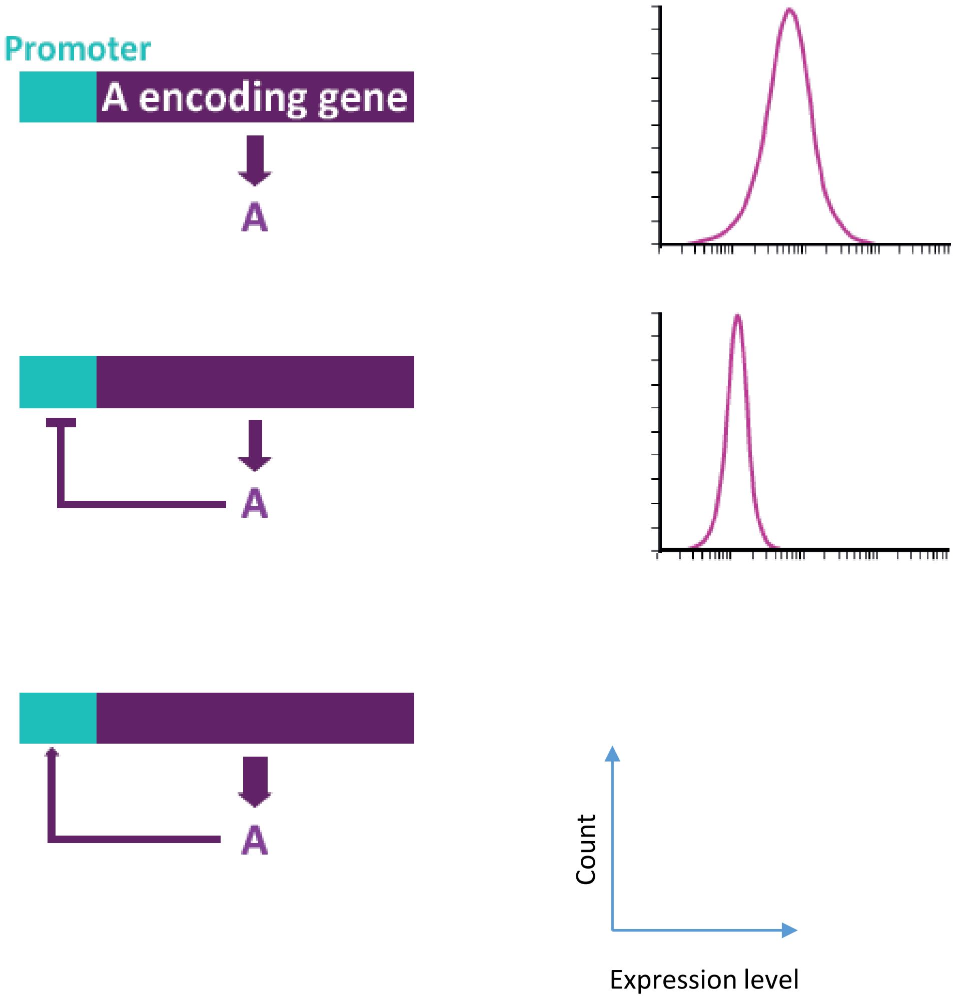
Regulatory networks influence gene expression noise



Constitutive unregulated expression

Negative feedback (autoregulation)

Weak positive feedback (autoregulation)



Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

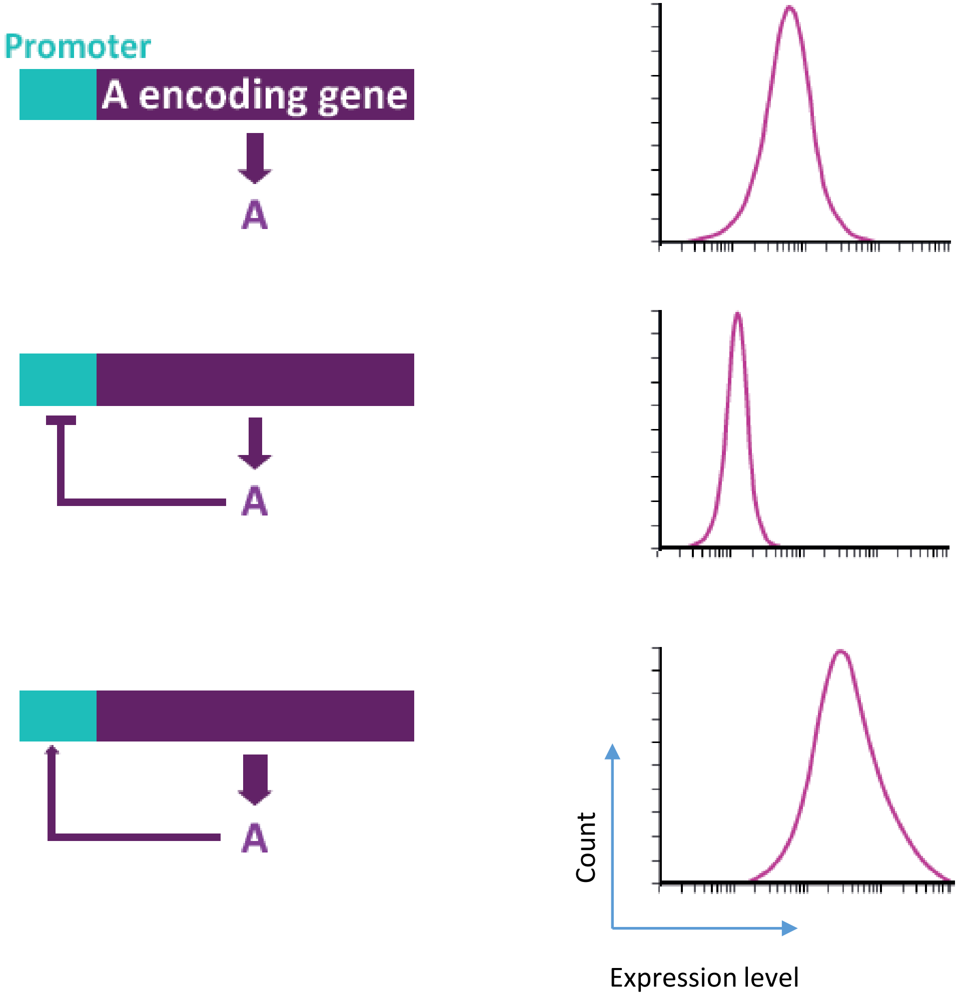
Noise is "controllable"

Regulatory networks influence gene expression noise

Constitutive unregulated expression

Negative feedback (autoregulation)

Weak positive feedback (autoregulation)



Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

Noise is "controllable"

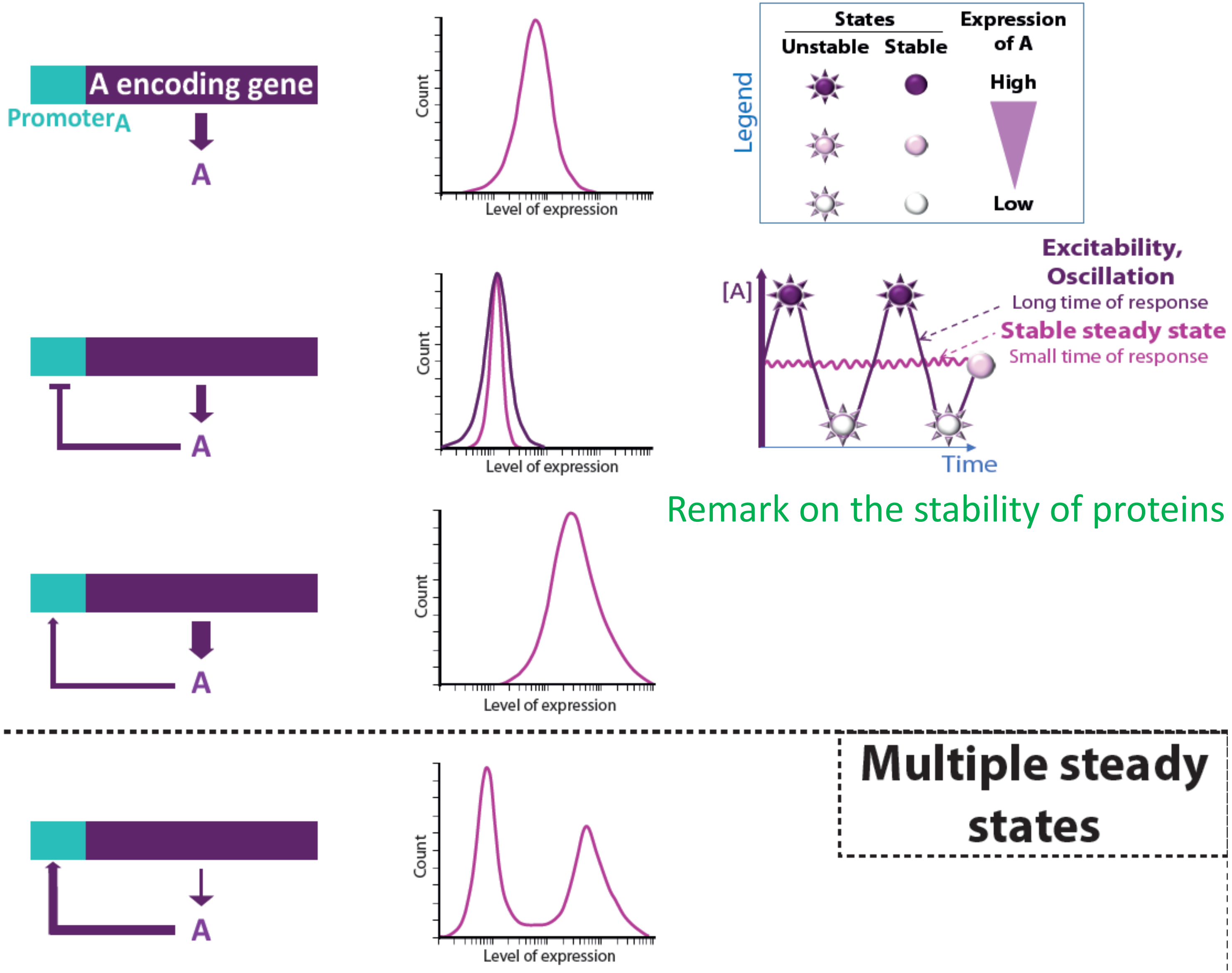
Regulatory networks influence gene expression noise

Constitutive unregulated expression

Negative feedback (autoregulation) or networks with odd numbers of negative feedback loops

Weak positive feedback (autoregulation)

Weak basal expression and Strong positive feedback (autoregulation)



Remark on the stability of proteins

Multiple steady states

Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

‘Activator + repressor’ modules coexistence can lead to bimodal expression patterns

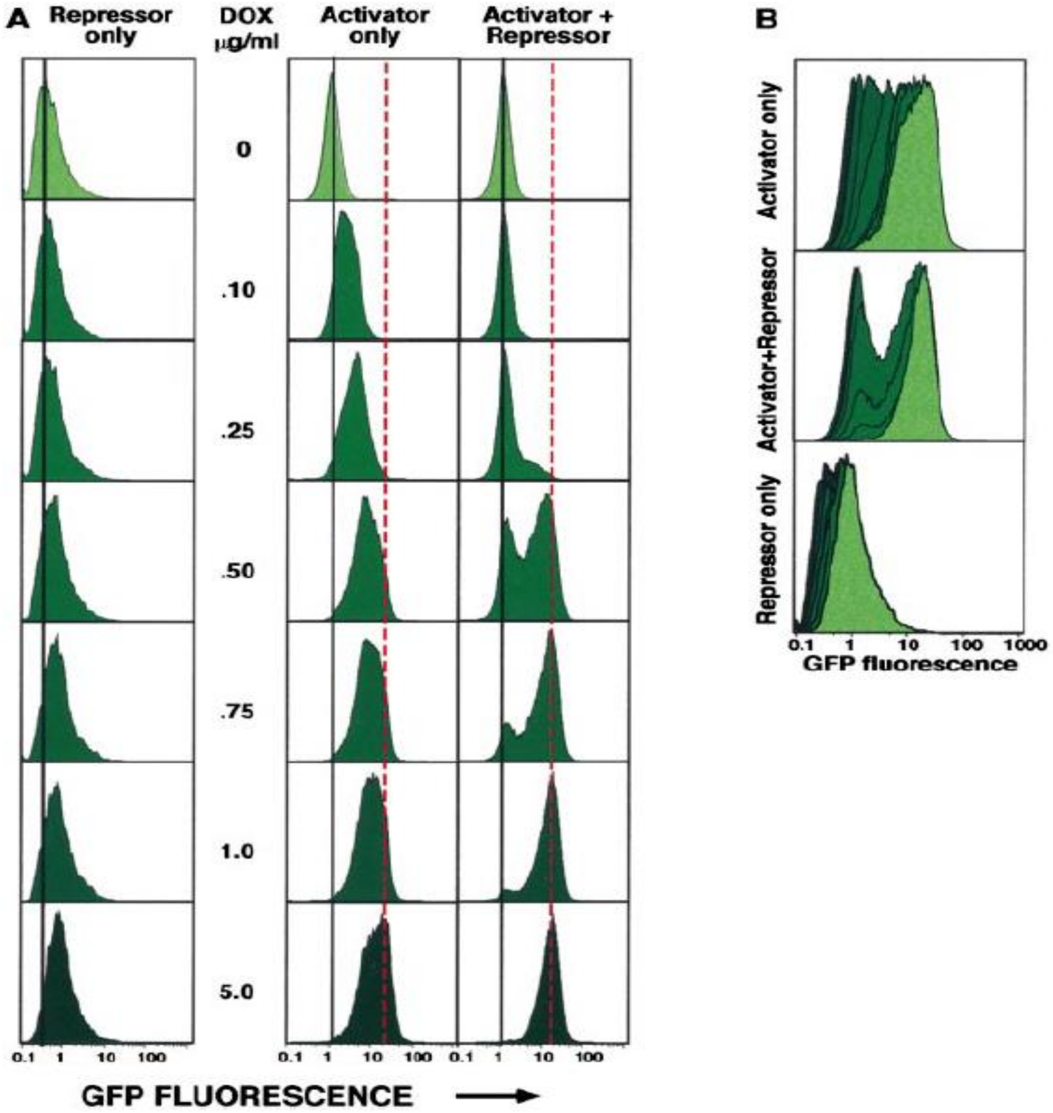


Figure 3. Single-Cell Analysis by Flow Cytometry

(A and B) The rheostat transcriptional response to increasing concentrations of the inducer mediated by either the activator or the repressor alone is converted to an on/off switch in single cells containing both factors. Populations of cells containing the repressor (“repressor only”), the activator (“activator only”), or both (“activator + repressor”) were treated for 72 hr with the concentrations of dox indicated. The distribution of GFP expression in the three populations was analyzed by flow cytometry. In (A), GFP expression profiles are shown for each population. The black and the red lines mark the positions of the peak of GFP expression in uninduced and induced conditions, respectively. In the repressor only and activator only populations, increasing concentrations of dox lead to a graded increase in GFP expression in the entire cell population, as indicated by a unimodal homogeneous shift to the right of the peak. In the activator + repressor population, increasing concentrations of dox lead to the expression of GFP in a subpopulation of the cells, while no GFP can be detected in the remainder of the population. This effect is indicated by the appearance of two distinct peaks in the GFP expression profiles (Figure 1C). A further increase in the dox concentration leads to an increase of the GFP-positive subpopulation at the expense of the GFP-negative subpopulation. The level of GFP expression in the positive subpopulation is equivalent at all doses of inducer, indicative of an all-or-none response. (B) shows an overlay of the GFP expression profiles shown in (A). Whereas in the repressor only and activator only populations a range of GFP expression levels can be achieved in response to changes in the concentration of the inducer, in the activator + repressor population GFP can either be absent or expressed at maximal levels, but cells expressing intermediate levels of GFP are rare.

Rossi *et al.*, *Molecular Cell* (2000)

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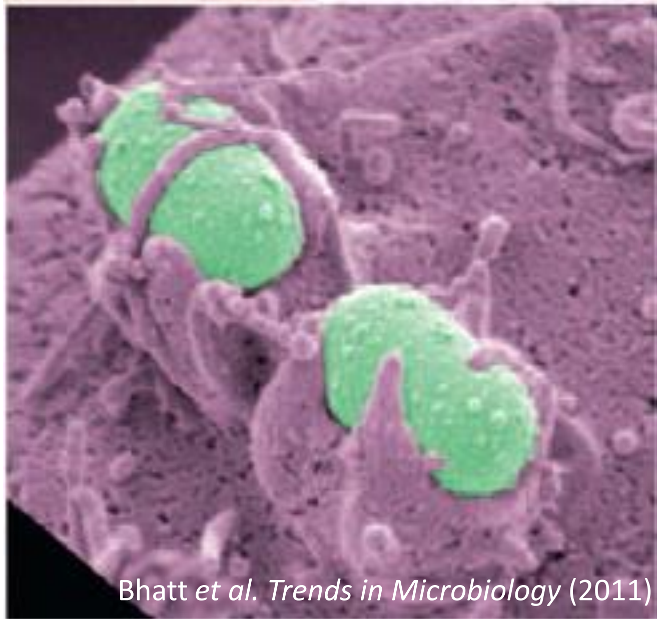
Noise and gene networks

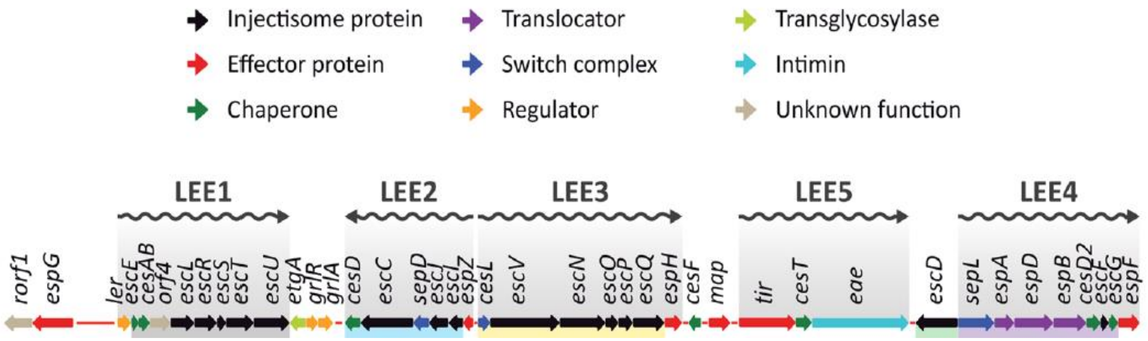
Example of bacterial virulence

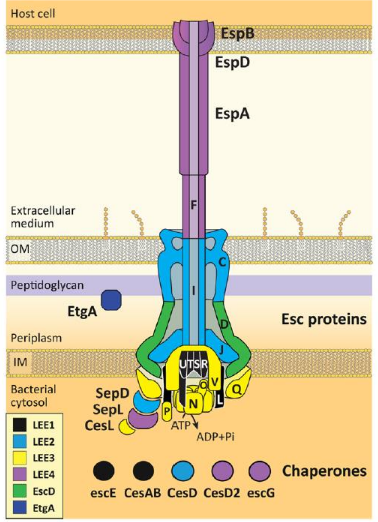
Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*



Bhatt *et al. Trends in Microbiology* (2011)

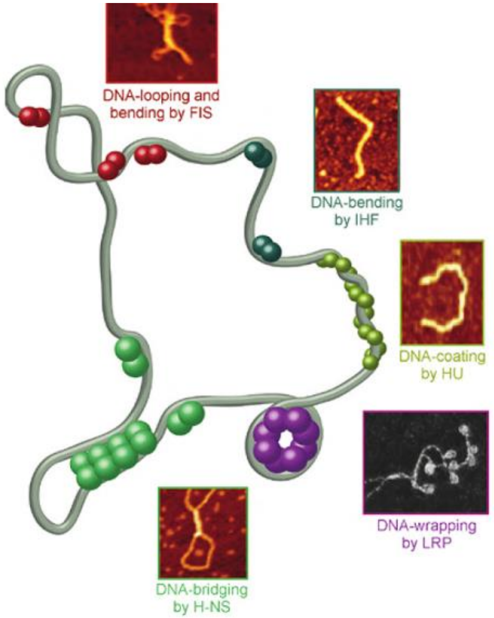
- A/E pathogens (attachment/effacement) : Enteropathogenic ***Escherichia coli* (EPEC)**, enterohemorragic (EHEC)…
- Phenotype A/E linked to the expression of the islet of pathogenicity LEE (locus of enterocyte effacement) encoding a type III secretion system



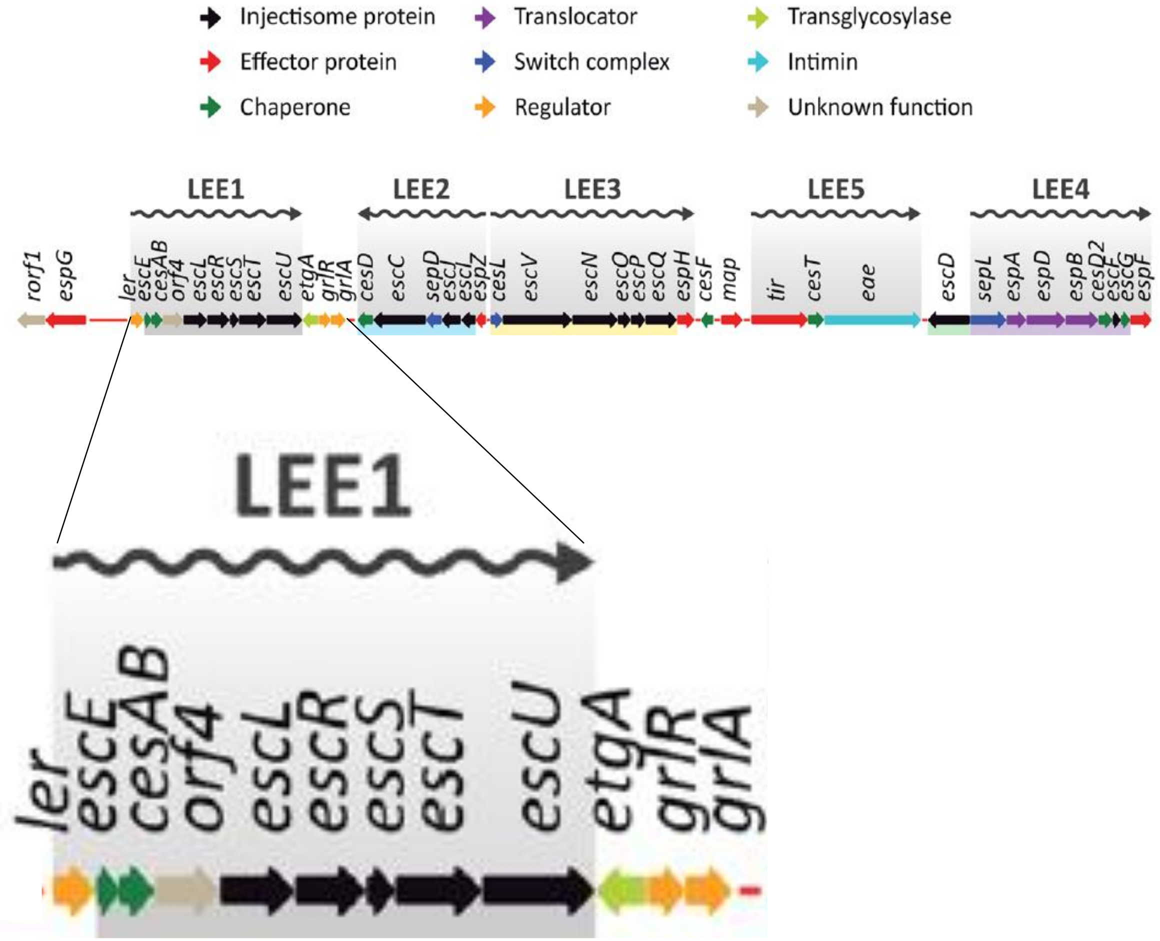


Ruano-Gallego *et al. ACS Synth Biol* (2015)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*



Pul & Wagner, in *Bacterial Chromatin* (Eds Springer, 2010)



Ler (***L**EE **e**ncoded **r**egulator*)

Belong to the xenogeneic silencers (H-NS family)

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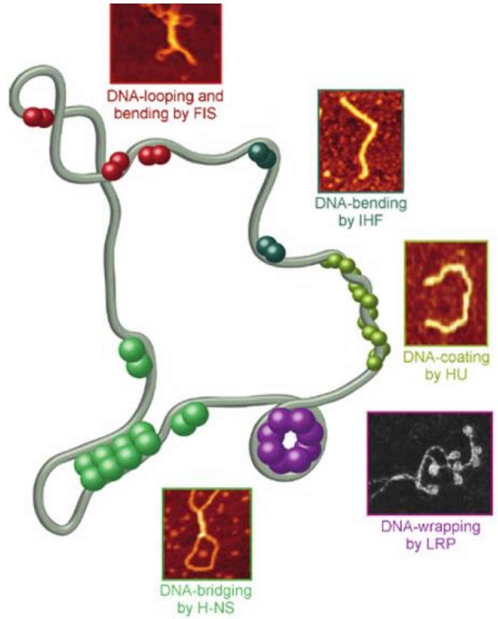
Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*



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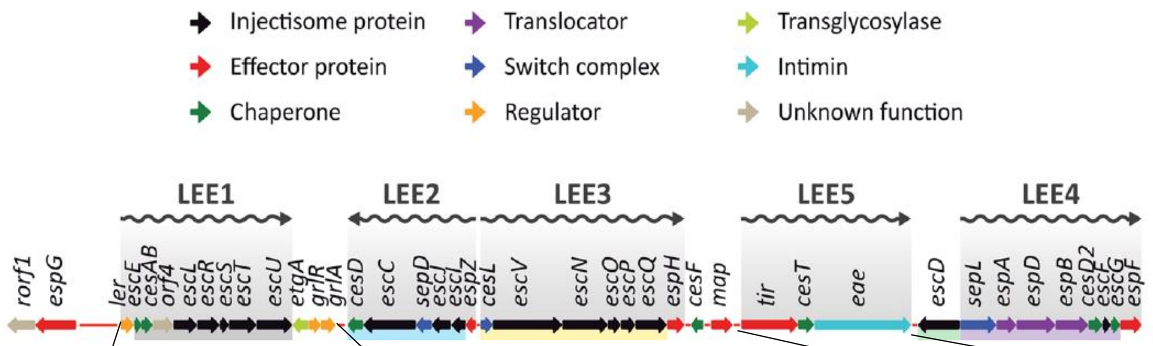

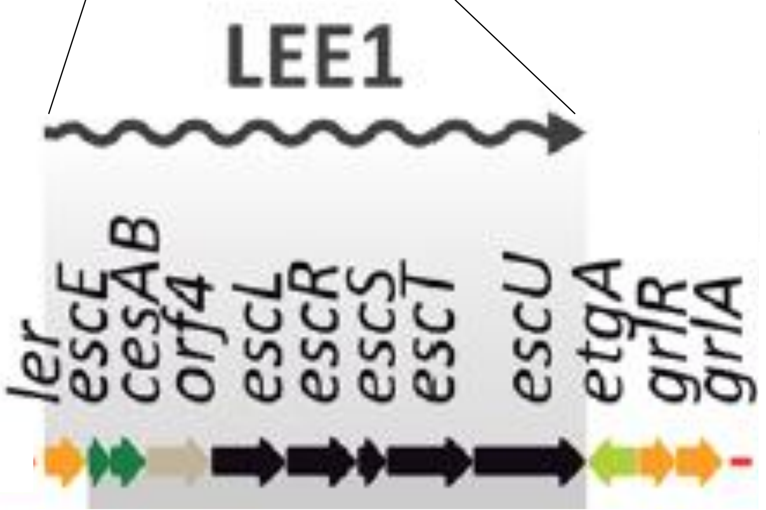

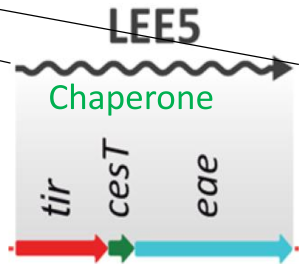

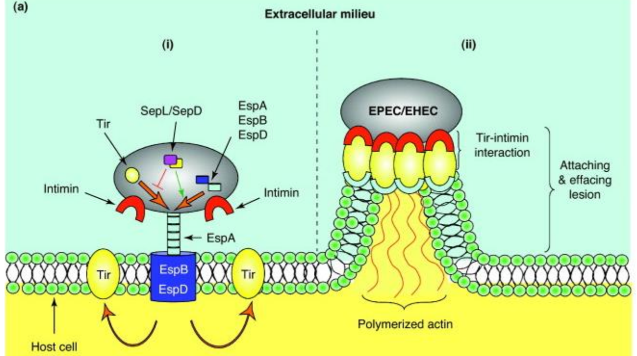

Bhatt *et al. Trends in Microbiology* (2011)

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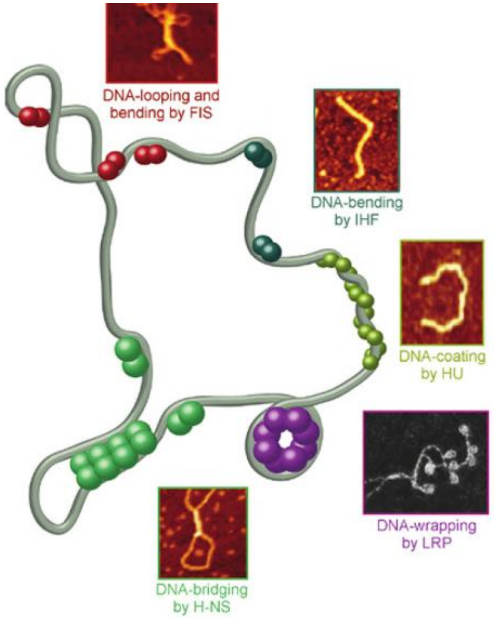
Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*



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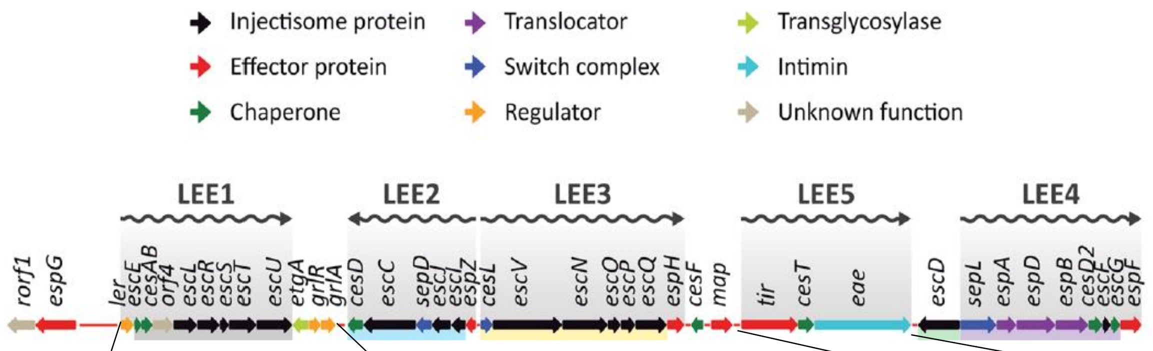
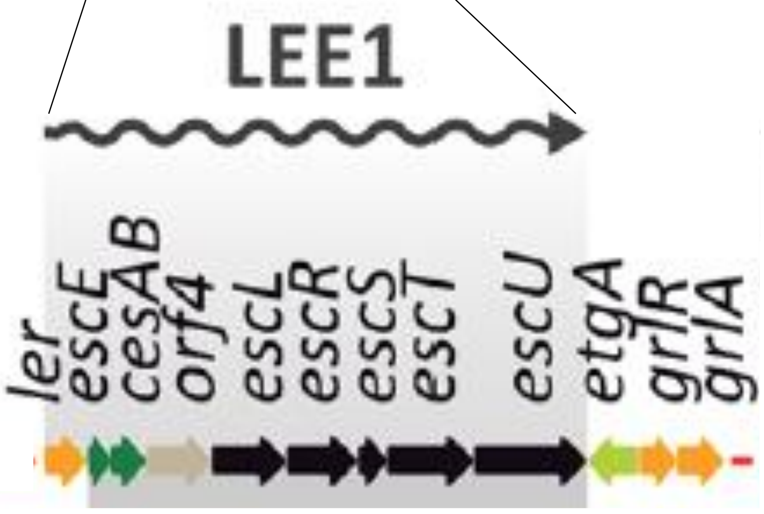
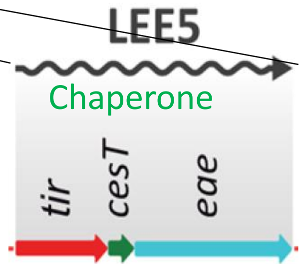




New game

4 EPEC strains:
- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$

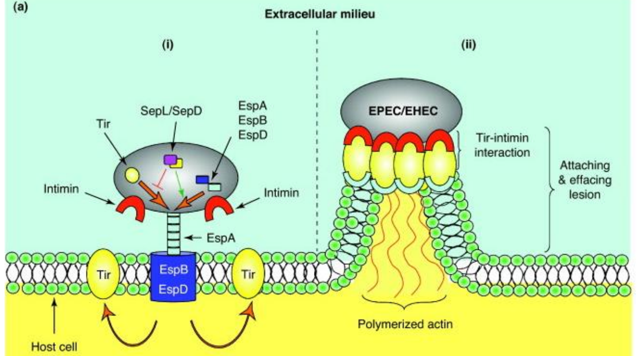
Prom$_{LEE5}$



Chaperone

Receptor

Intimine/Adhesin



Bhatt *et al. Trends in Microbiology* (2011)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

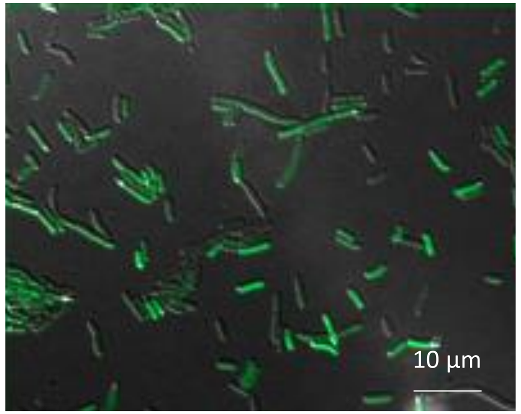
New game

4 EPEC strains:
- WT
- *hns⁻*
- *ler⁻*
- *hns⁻ler⁻*

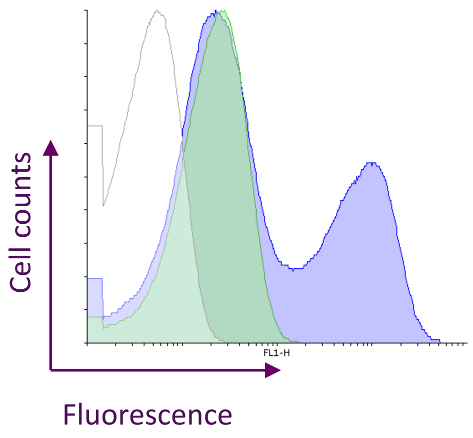
Prom$_{LEE5}$

EPEC negative control: no *gfp* gene

LB = medium considered as a **non- inducing** condition



LB medium



Yes

No

① Is the pathogenicity island expressed under non-inducing condition?

Leh *et al. mBIO* (2017)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

New game

4 EPEC strains:
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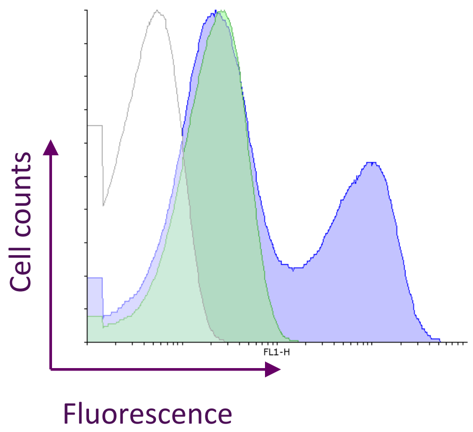
- *hns*$^-$*ler*$^-$

Prom$_{LEE5}$

EPEC negative control: no *gfp* gene

LB medium



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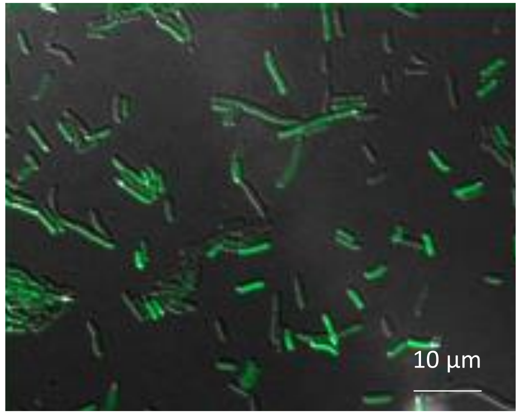

① Yes, the pathogenicity island expressed under non-inducing condition!

Bet-hedging theory

Leh *et al. mBIO* (2017)

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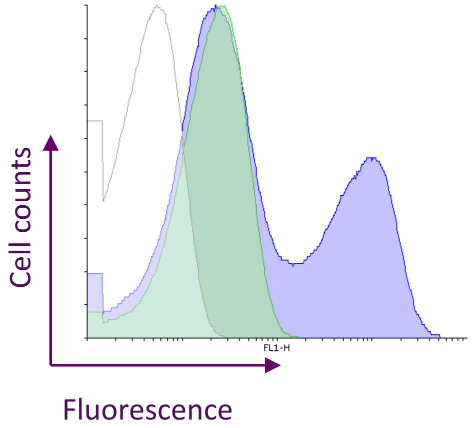
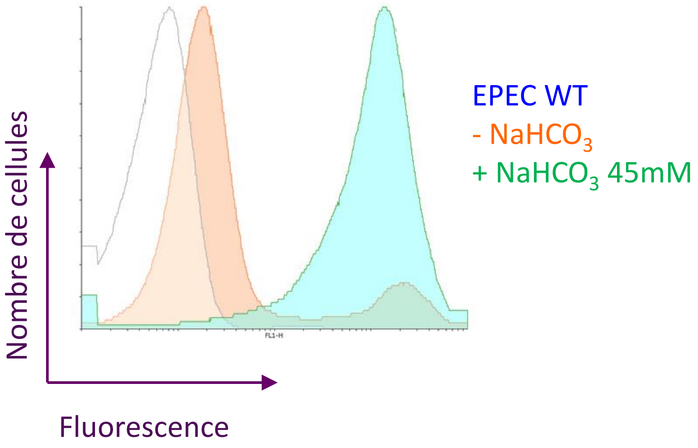
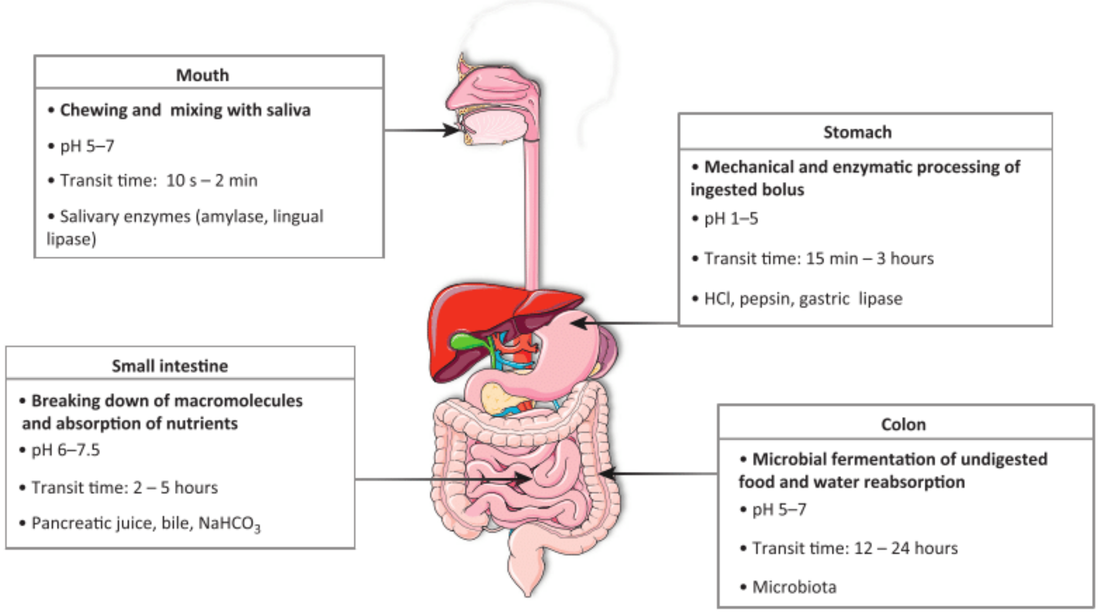
LB medium



SF93M medium





Mouth
- **Chewing and mixing with saliva**
- pH 5–7
- Transit time: 10 s – 2 min
- Salivary enzymes (amylase, lingual lipase)

Stomach
- **Mechanical and enzymatic processing of ingested bolus**
- pH 1–5
- Transit time: 15 min – 3 hours
- HCl, pepsin, gastric lipase

Small intestine
- **Breaking down of macromolecules and absorption of nutrients**
- pH 6–7.5
- Transit time: 2 – 5 hours
- Pancreatic juice, bile, $NaHCO_3$

Colon
- **Microbial fermentation of undigested food and water reabsorption**
- pH 5–7
- Transit time: 12 – 24 hours
- Microbiota

Leh *et al. mBIO* (2017)

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Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

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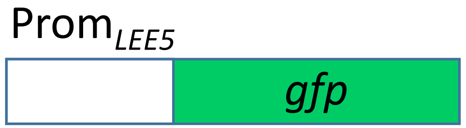
- *hns*$^-$*ler*$^-$



EPEC negative control: no *gfp* gene

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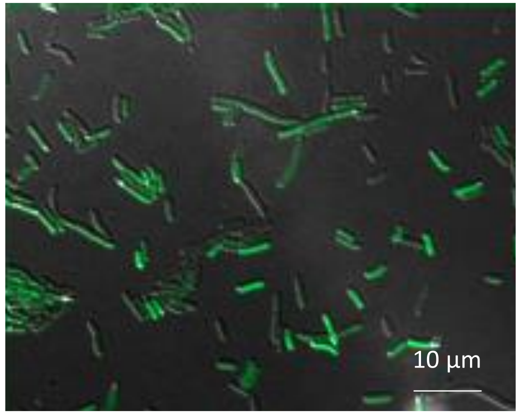
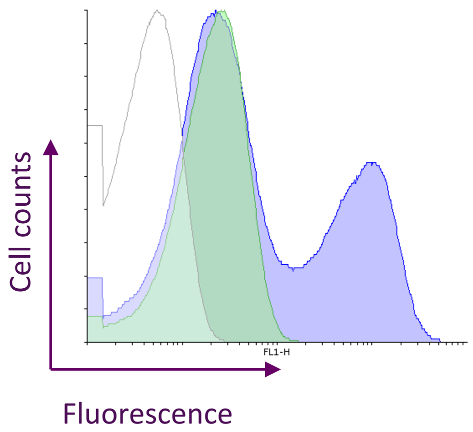


LB medium



② Effect of Ler xenogeneic silencer on *LEE5* promoter ? (activator or silencer)

🟦 👍

🟪 👎

Leh *et al. mBIO* (2017)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

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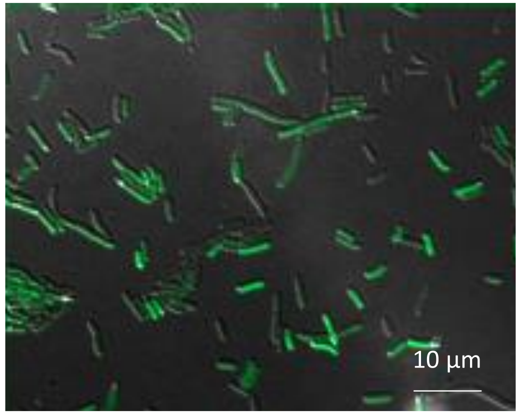
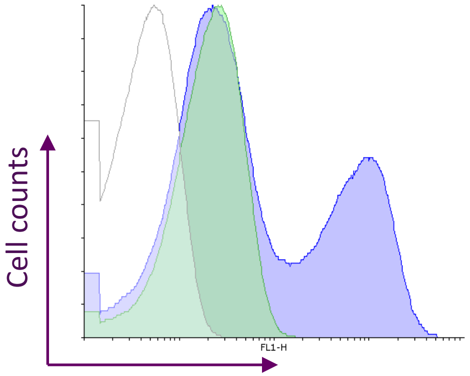


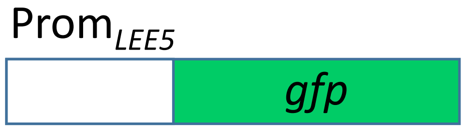
LB medium



Fluorescence

New game

4 EPEC strains:
- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$



EPEC negative control: no *gfp* gene

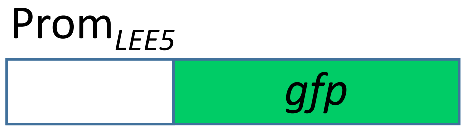
② Ler is an activator!

Leh *et al.* *mBIO* (2017)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

New game

4 EPEC strains:
- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$



EPEC negative control: no *gfp* gene

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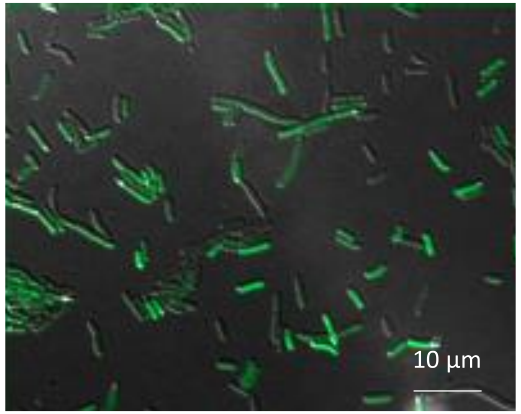
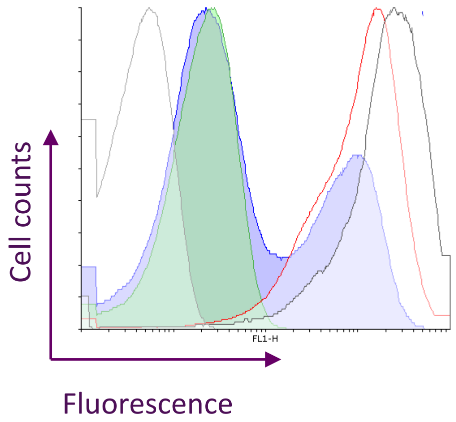


LB medium



③ Effect of H-NS xenogeneic silencer on *LEE5* promoter ? (activator or silencer)

🟦 🟪

👍 👎

Leh *et al. mBIO* (2017)

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Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

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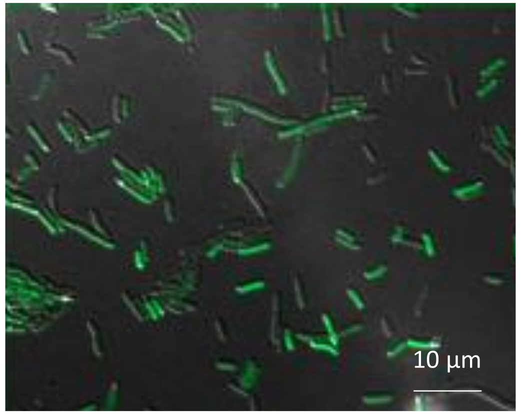
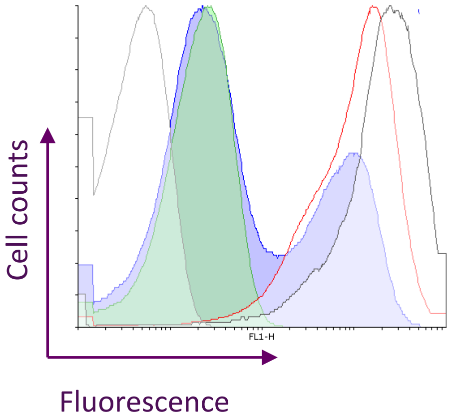


LB medium



New game

4 EPEC strains:
- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$

Prom$_{LEE5}$

EPEC negative control: no *gfp* gene

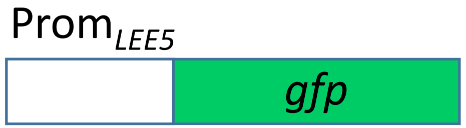
③ H-NS silences *LEE5* promoter.

Leh *et al. mBIO* (2017)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

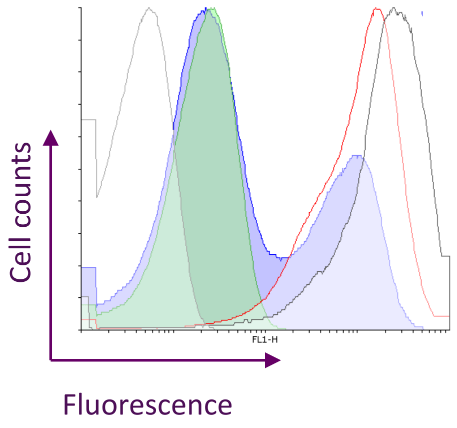
New game

4 EPEC strains:
- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$



EPEC negative control: no *gfp* gene

LB medium



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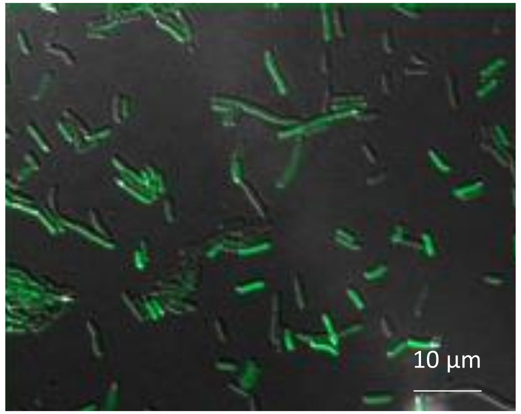

④ Effect of Ler on *LEE5* promoter in absence of H-NS? (activator or silencer)

🟦 🟪

👍 👎

Leh *et al. mBIO* (2017)

Nucleoid associated proteins and regulation of the expression of a pathogenicity island in *Escherichia coli*

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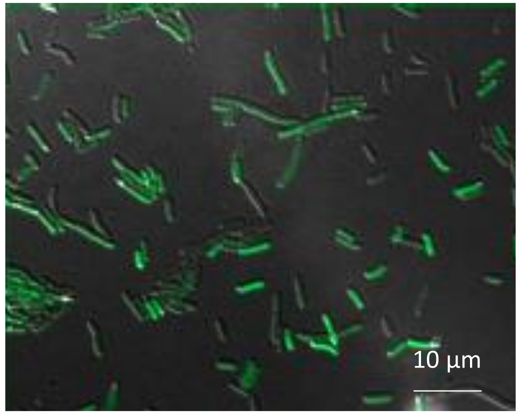

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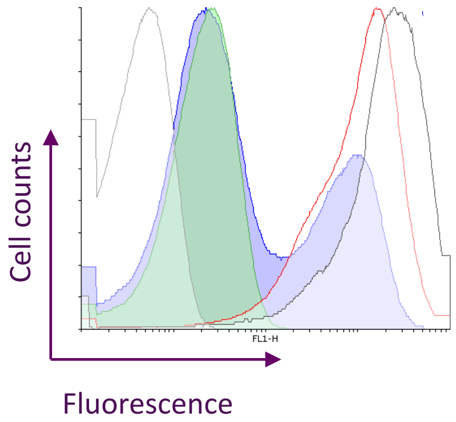
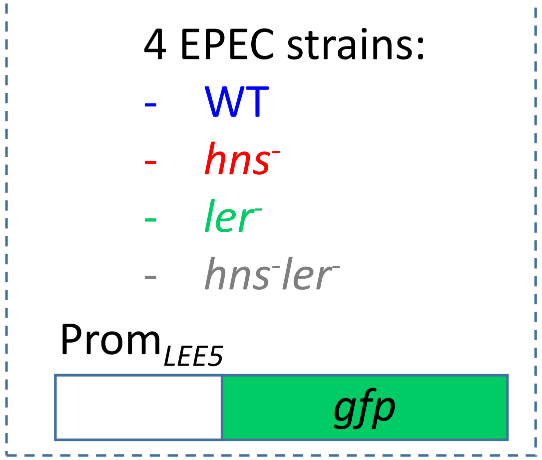


New game



4 EPEC strains:

- WT
- *hns*$^-$
- *ler*$^-$
- *hns*$^-$*ler*$^-$

EPEC negative control: no *gfp* gene

④ Ler is not required for LEE5 activation in absence of H-NS!

=> Regulation model?

Leh *et al. mBIO* (2017)

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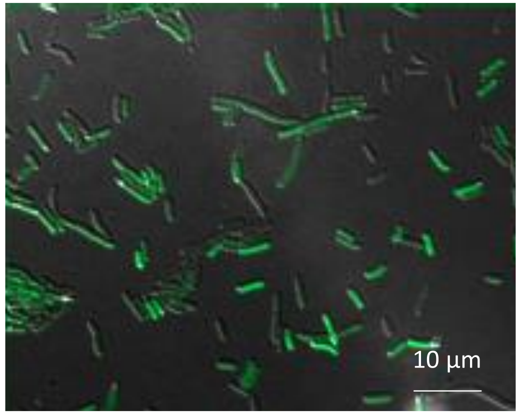

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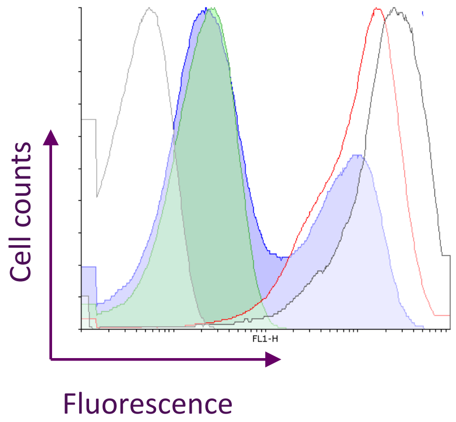

New game

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- WT
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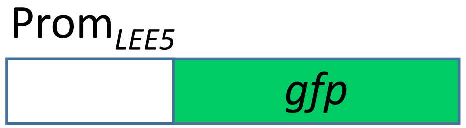
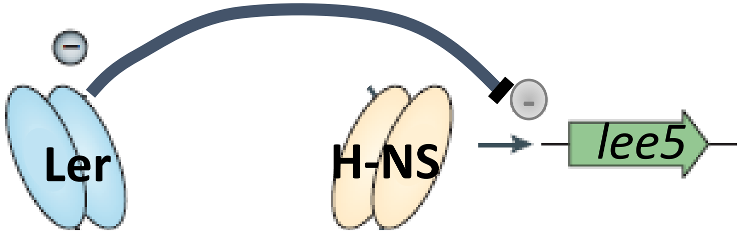
- *hns*$^-$*ler*$^-$



EPEC negative control: no *gfp* gene

Relief of H-NS mediated repression

Only partial in LB
Complete under activating conditions

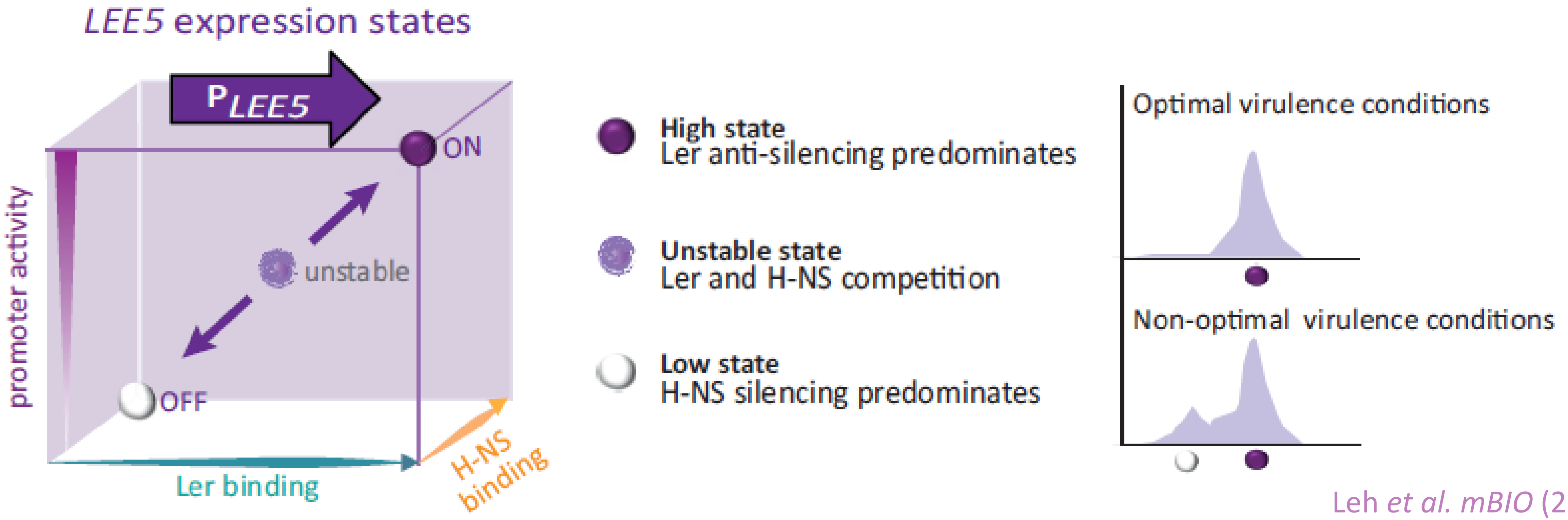


Leh *et al. mBIO* (2017)

Model of *LEE5* promoter regulation that can result in a bimodal population

Is bimodal expression found in other pathogens (e.g. *Vibrio cholerae, Samonella*...) which pathogenicity island is also silenced by H-NS?

Link between the structure of the bacterial chromatin and bimodality!



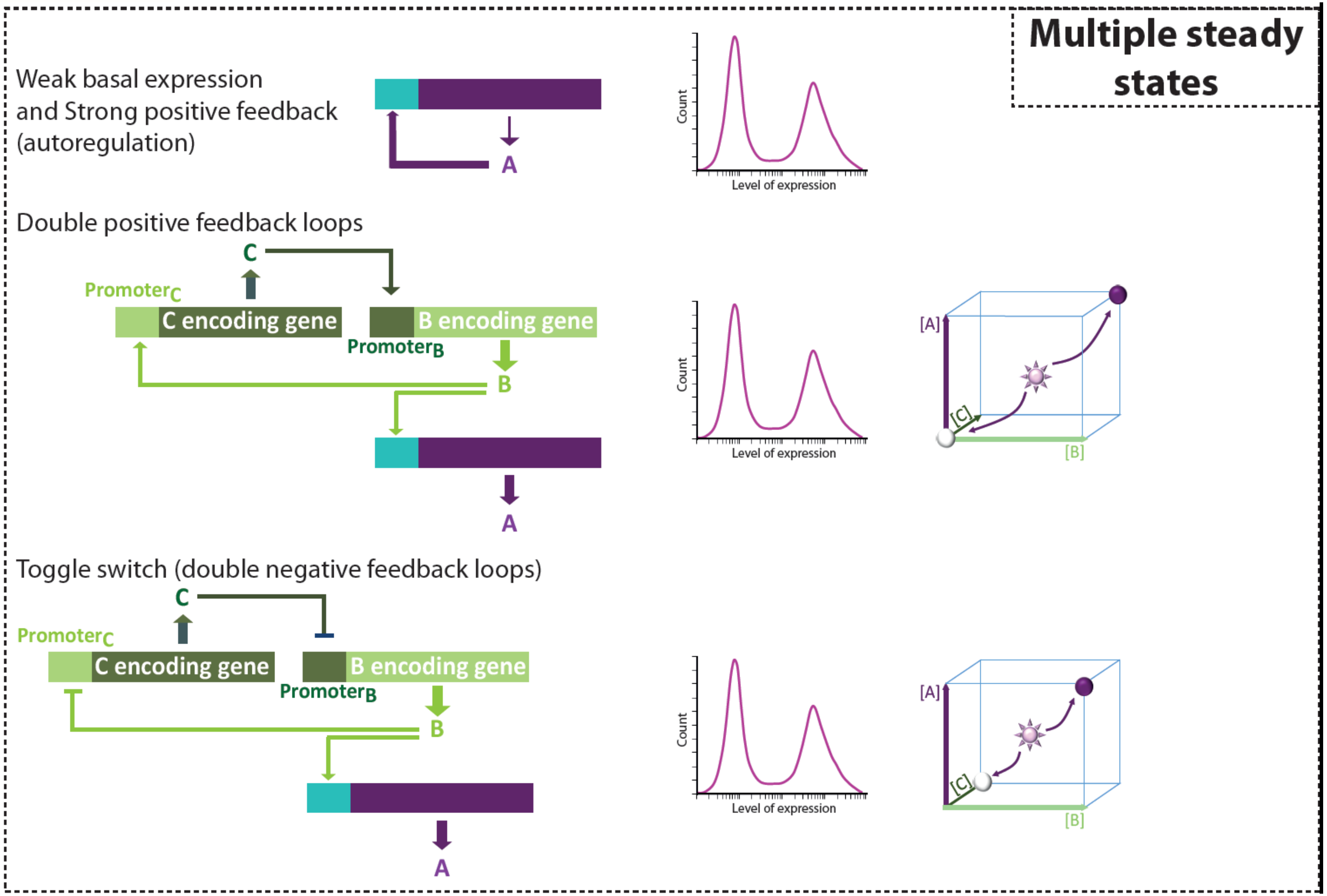
● **High state**
Ler anti-silencing predominates

● **Unstable state**
Ler and H-NS competition

○ **Low state**
H-NS silencing predominates

Leh *et al. mBIO* (2017)

Regulation networks likely to generate bimodality

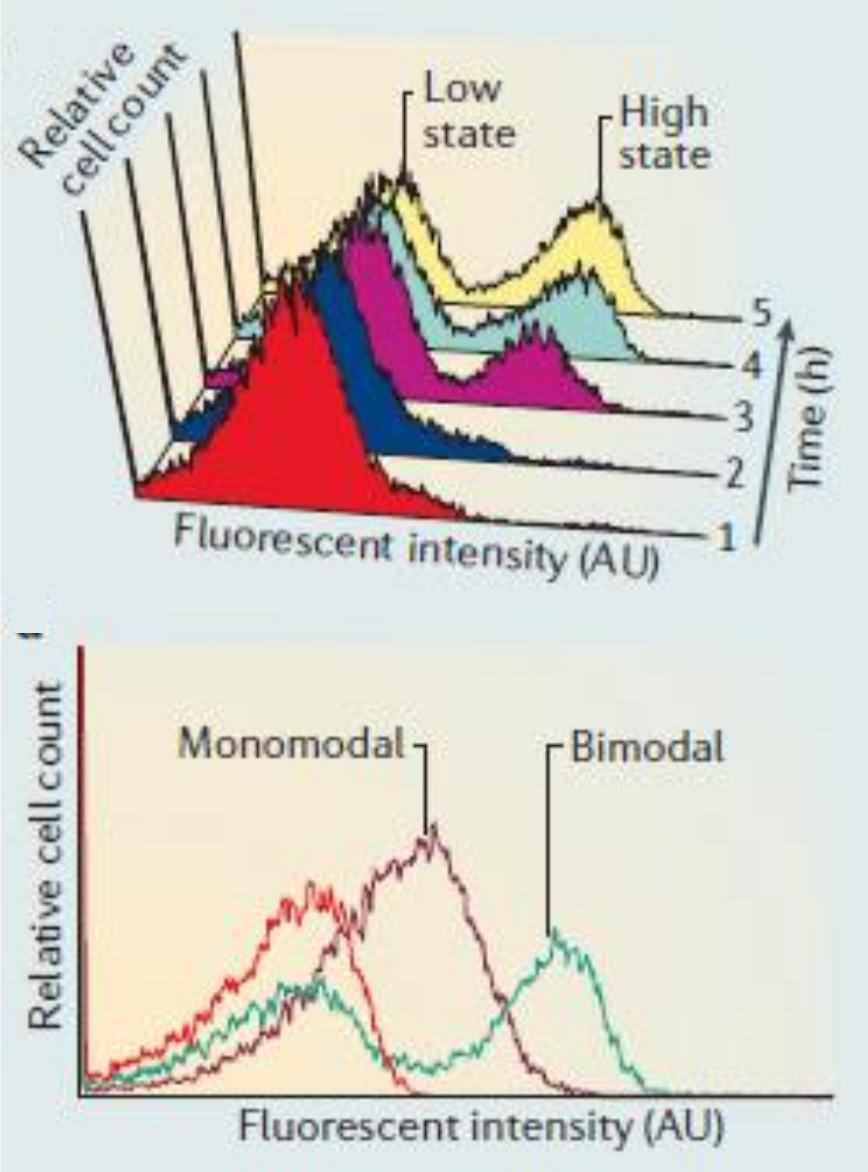


Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

> « Bistability is impossible if one of the components acts too strongly or too weakly compared to the others. »

Smith *et al.*, *Nature Reviews Microbiology* (2006)

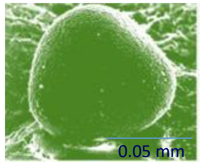
Stochasticity and bimodality



Smith *et al.*, *Nature Reviews Microbiology* (2006)

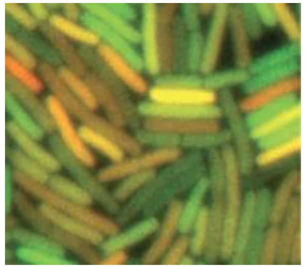
Examples

Competence *(Bacillus subtilis, Streptococcus pneumoniae)*
Lactose operon (*Escherichia coli*)
Lytic *vs.* Lysogenic decision during λ phage infection
SPI secretion system (*Salmonella*)
Cytotoxicity & mucoidy (*Pseudomonas aeruginosa)*
Sporulation (*Bacillus subtilis)*
Formation of the fruiting body (*Mycococcus xanthus*)

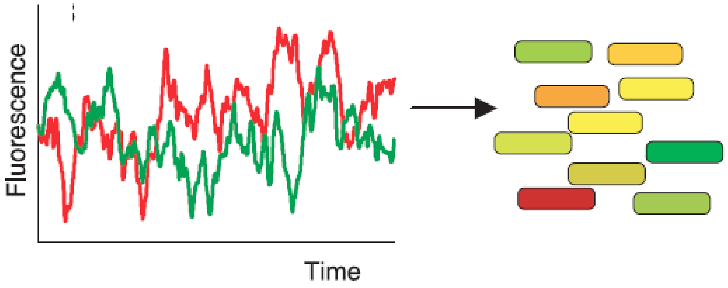


Kaiser, TIG (1999)

The stochasticity of gene expression is considered to be systematic.
The plurimodal nature is more specific to certain genes and conditions.



Elowitz *et al.*, *Science* (2002)



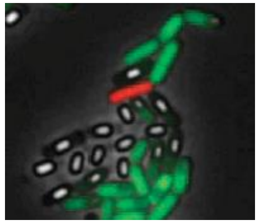
Metabolism

(e.g. Lactose operon/*Escherichia coli*)

Stress response

Competence (e.g. *Bacillus subtilis*)
Sporulation (e.g. *Bacillus subtilis*)
Persistence (e.g. *Staphylococcus aureus*)

Clonal population of Bacillus subtilis in a state of vegetative growth or in the process of sporulation (green), at the end of sporulation (white) or in a state of competence (red)



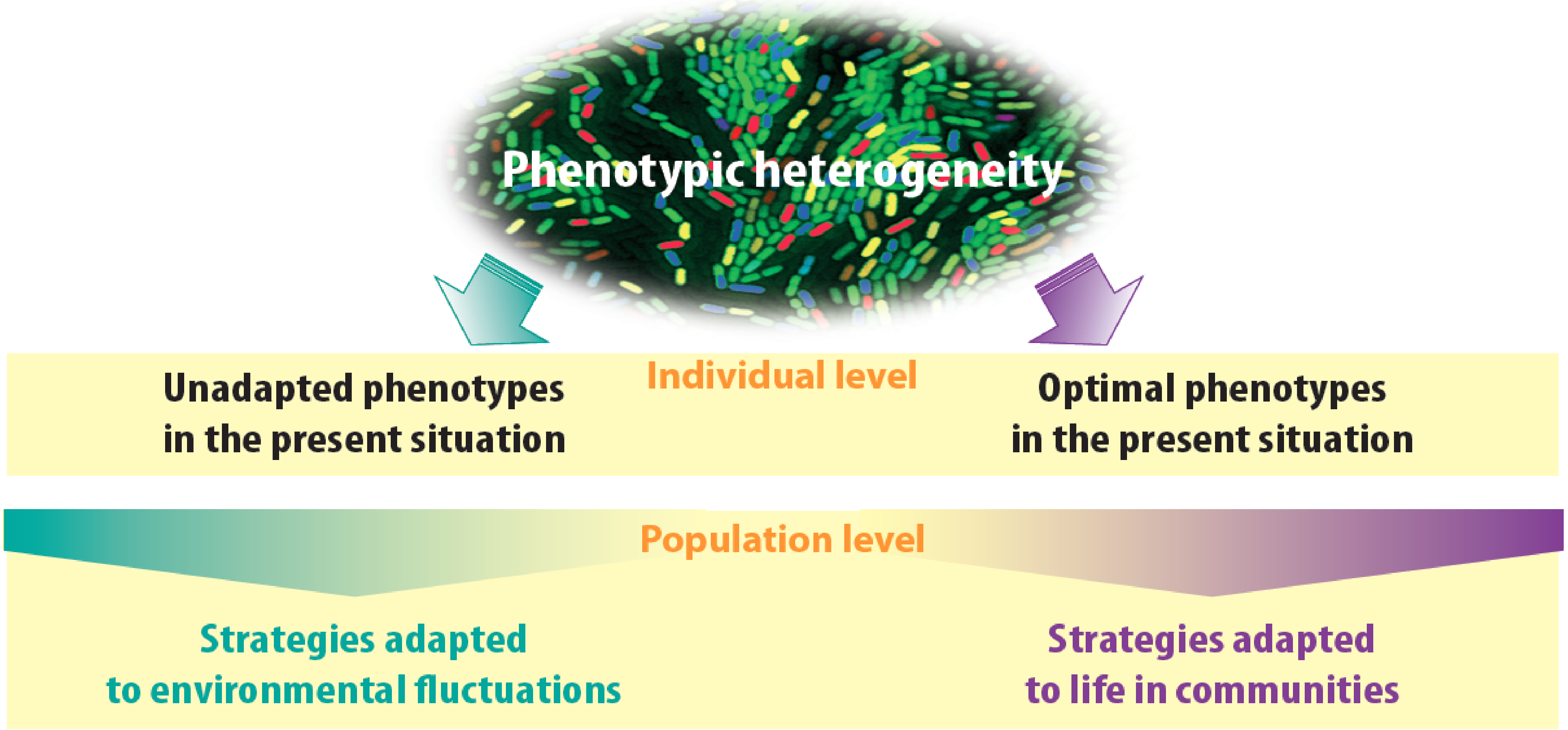
Eldar & Elowitz, *Nature* (2010)

Pathogenicity

Mucoidy
(e.g. *Pseudomonas aeruginosa*)
Defense *vs* offensive mode (e.g. *S. aureus*)
Expression *vs* latency (e.g. HIV)

Conclusions & Perspectives

> ***Every man knows how useful it is to be useful. No one seems to know how useful it is to be useless.***
>
> Tchouang-Tseu



Phenotypic heterogeneity

Individual level

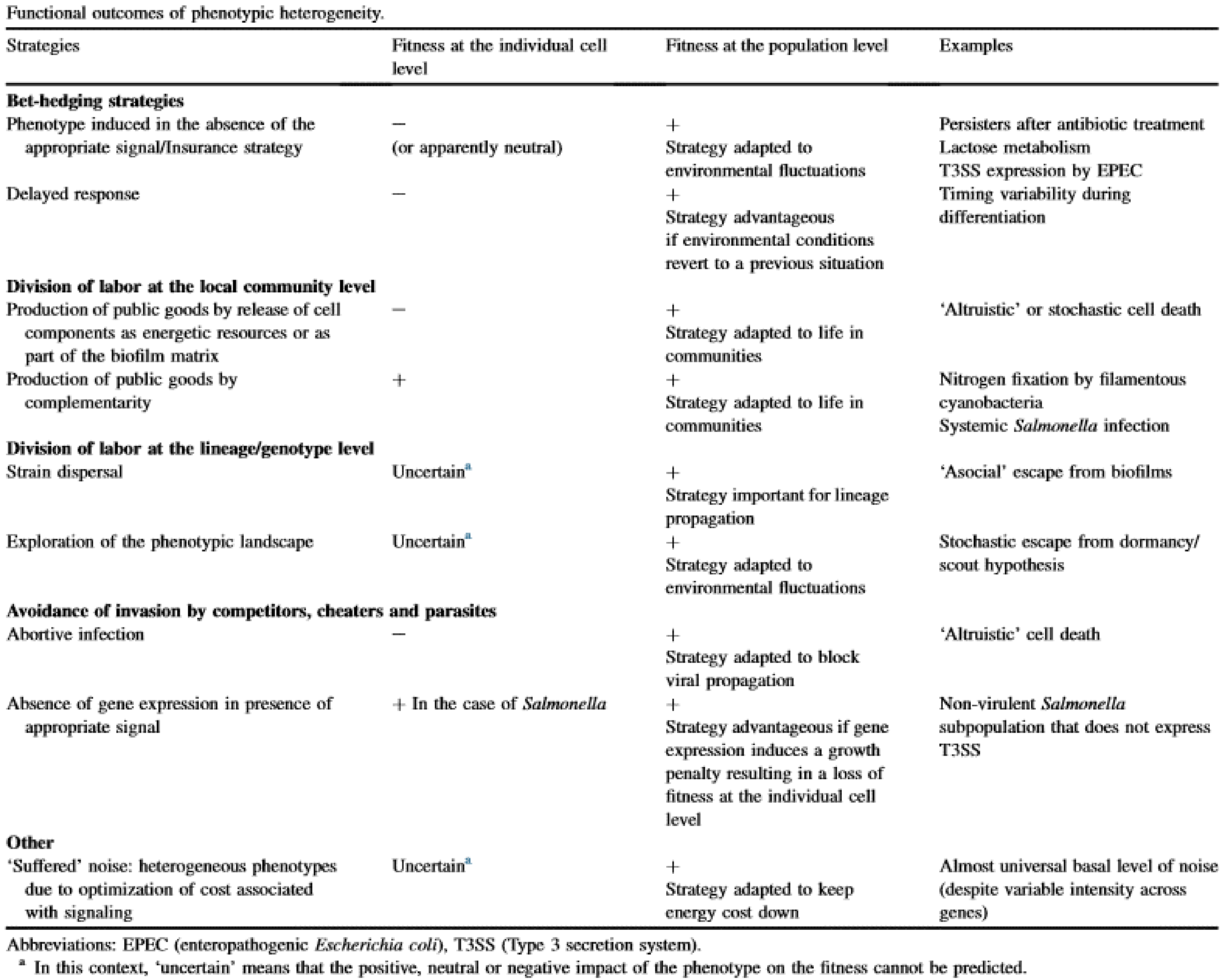
Unadapted phenotypes in the present situation

Optimal phenotypes in the present situation

Population level

Strategies adapted to environmental fluctuations

Strategies adapted to life in communities



Functional outcomes of phenotypic heterogeneity.

Strategies	Fitness at the individual cell level	Fitness at the population level	Examples
Bet-hedging strategies			
Phenotype induced in the absence of the appropriate signal/Insurance strategy	− (or apparently neutral)	+ Strategy adapted to environmental fluctuations	Persisters after antibiotic treatment Lactose metabolism T3SS expression by EPEC
Delayed response	−	+ Strategy advantageous if environmental conditions revert to a previous situation	Timing variability during differentiation
Division of labor at the local community level			
Production of public goods by release of cell components as energetic resources or as part of the biofilm matrix	−	+ Strategy adapted to life in communities	'Altruistic' or stochastic cell death
Production of public goods by complementarity	+	+ Strategy adapted to life in communities	Nitrogen fixation by filamentous cyanobacteria Systemic *Salmonella* infection
Division of labor at the lineage/genotype level			
Strain dispersal	Uncertain[a]	+ Strategy important for lineage propagation	'Asocial' escape from biofilms
Exploration of the phenotypic landscape	Uncertain[a]	+ Strategy adapted to environmental fluctuations	Stochastic escape from dormancy/scout hypothesis
Avoidance of invasion by competitors, cheaters and parasites			
Abortive infection	−	+ Strategy adapted to block viral propagation	'Altruistic' cell death
Absence of gene expression in presence of appropriate signal	+ In the case of *Salmonella*	+ Strategy advantageous if gene expression induces a growth penalty resulting in a loss of fitness at the individual cell level	Non-virulent *Salmonella* subpopulation that does not express T3SS
Other			
'Suffered' noise: heterogeneous phenotypes due to optimization of cost associated with signaling	Uncertain[a]	+ Strategy adapted to keep energy cost down	Almost universal basal level of noise (despite variable intensity across genes)

Abbreviations: EPEC (enteropathogenic *Escherichia coli*), T3SS (Type 3 secretion system).

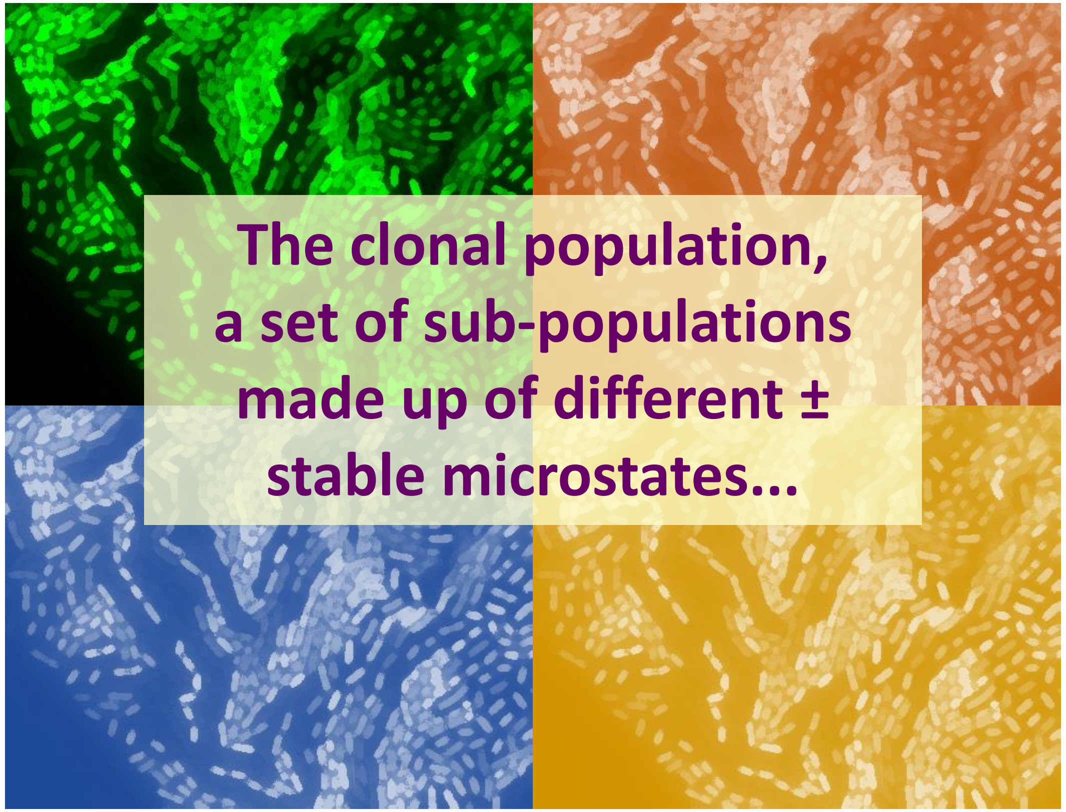
[a] In this context, 'uncertain' means that the positive, neutral or negative impact of the phenotype on the fitness cannot be predicted.

Bury-Moné & Sclavi, *Res. Microbiol.* (2017)

The noise of gene expression is :
- "Universal"
- Genetically coded
- A trait subject to evolution / selection
- "Controllable": Sensitive to the structure of regulatory networks capable of generating changes in life states without recourse to genetic mutation.

$\Rightarrow$ **Genetically inherited**
$\Rightarrow$ **Epigenetics? Memory ?**

Stochasticity of gene expression, another look at unicellulars...



The clonal population, a set of sub-populations made up of different ± stable microstates...

Single cells...

... whose "raison d'être" often finds a "meaning" / selective advantage at the evolutionary level at the population level and not necessarily at the individual level.

Sociomicrobiology
Microbial community
« Multicellularity »

What is the link between stochasticity and « Cellular innovations and synthetic microbiology concepts »?

- Fitness at the population level
- Emergence of new properties at the population level
- Link with synthetic biology :
  - Noise as a potential barrier to the design of controlled tools,
  - Noise as the possibility of generating genetic constructs with "controlled" stochasticity of gene expression.
  - Interest of synthetic constructs to study noise.

The question remains as to whether the biological processes described are in themselves stochastic or appear stochastic to us.