IV Master in Biophysics
Universidad Autónoma de Madrid
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Stochastic dynamics

Evolutionary Systems Biology Lab

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day III


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Stochastic dynamics of gene expression, experiments!

-Intrinsic noise in *Bacillus subtilis*

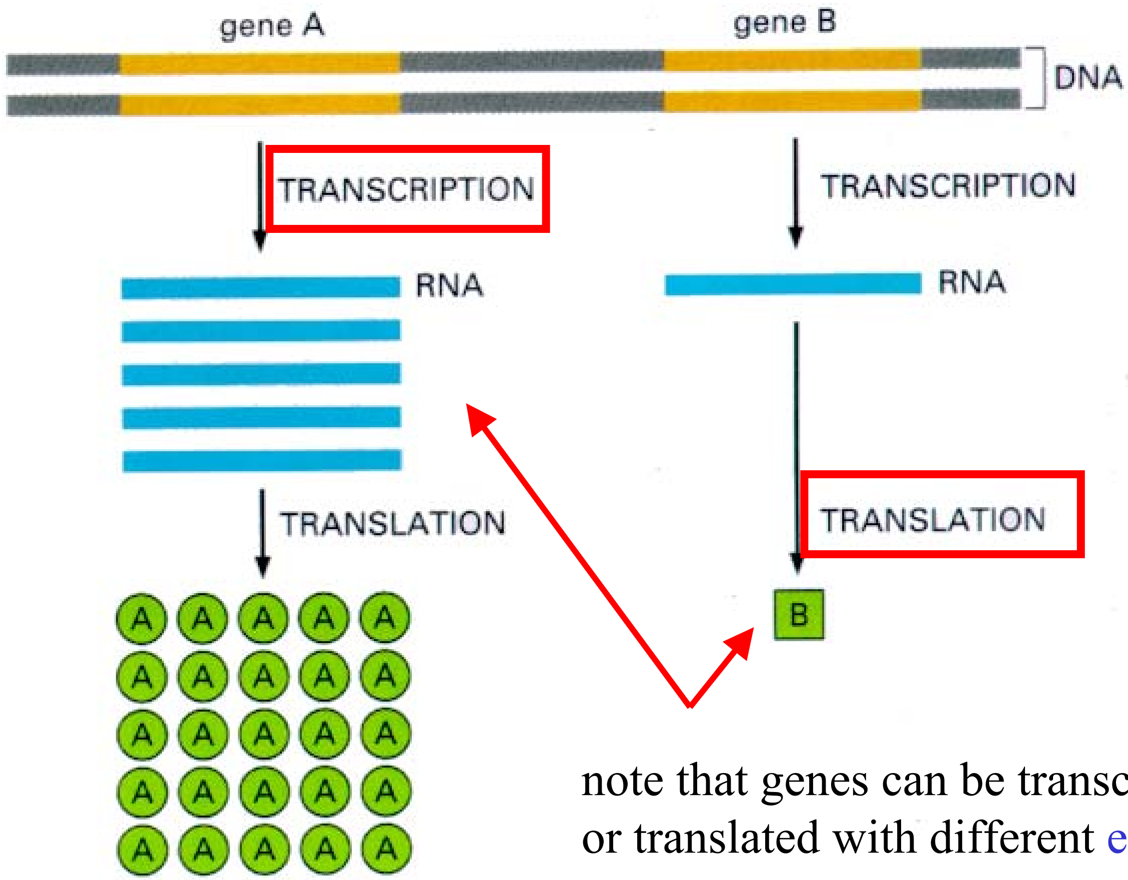
Molecular fluctuations within single cells (biochemical noise) →
→ variability in a genetically identical population (phenotypic noise).

- Extrinsic vs. intrinsic noise in *Escherichia coli*

Detection of noise and discrimination between intrinsic and extrinsic noise

Intrinsic noise in *Bacillus subtilis*

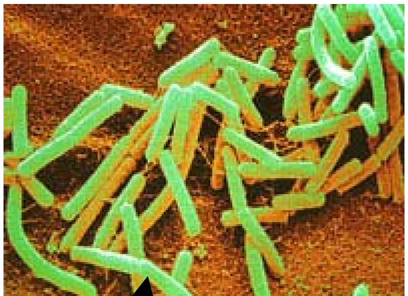
Gene expression in a nutshell

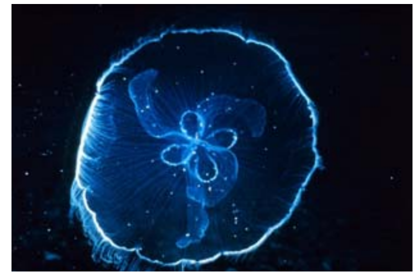


note that genes can be transcripted
or translated with different efficiencies/rates

Intrinsic noise in *Bacillus subtilis*

- A single-copy chromosomal gene with an inducible promoter was introduced in *B. subtilis*



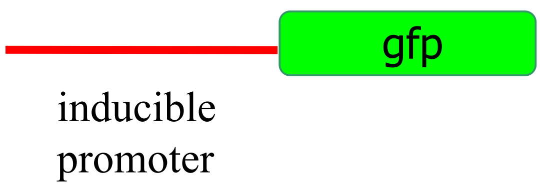


gfp

- protein from **jellyfish** that fluoresces green when exposed to light.

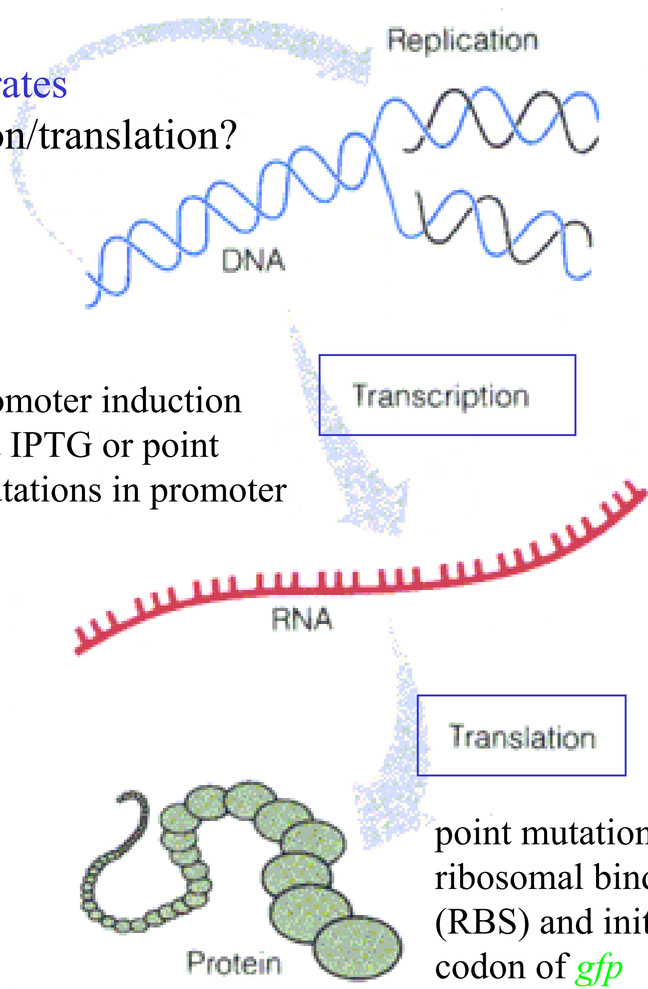
- one can take this protein and express it in a different organism (genetic engineering)

- great tool for studies of genetic networks!



Intrinsic noise in *Bacillus subtilis*

How to vary rates
of transcription/translation?

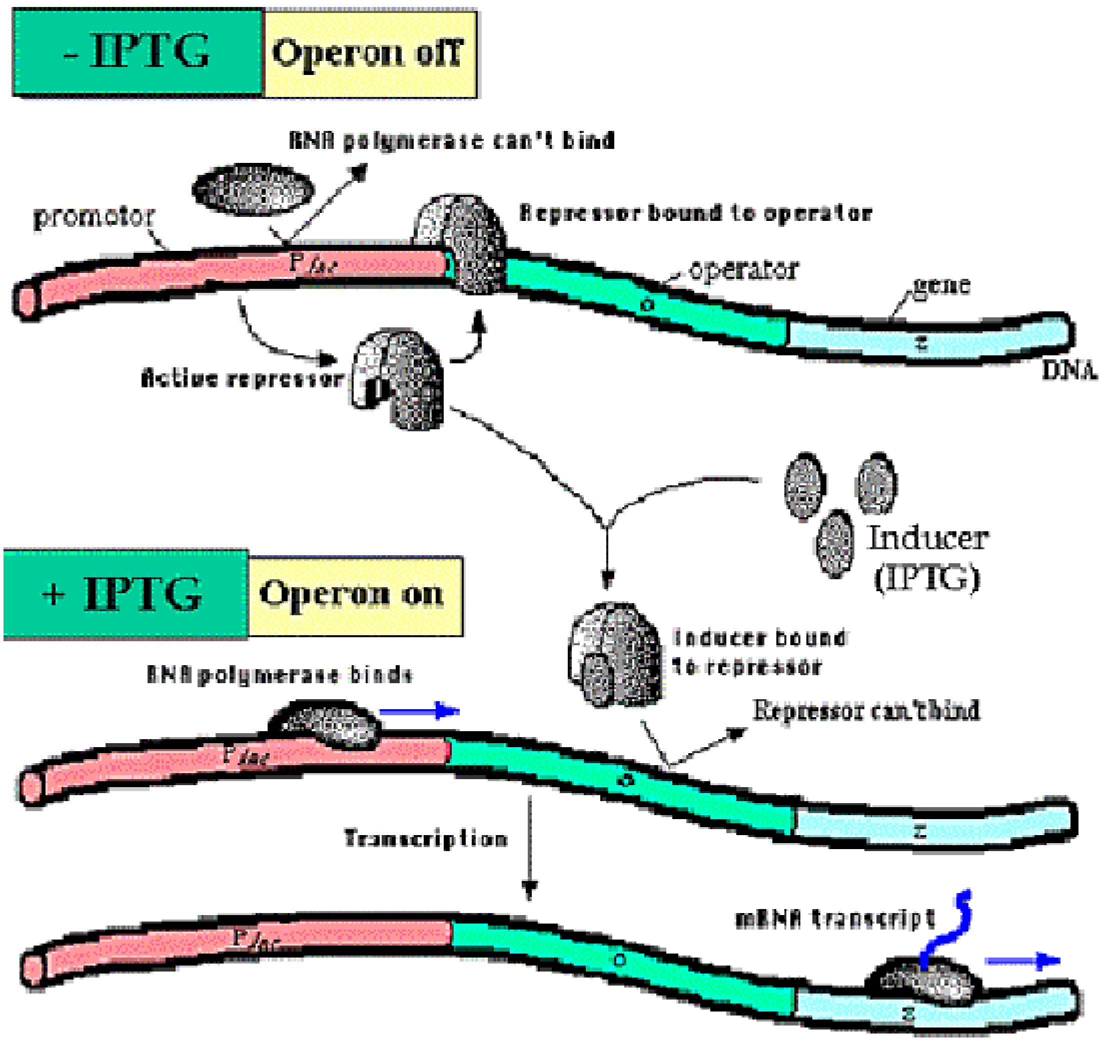


promoter induction
via IPTG or point
mutations in promoter

point mutations in
ribosomal binding site
(RBS) and initiation
codon of *gfp*

Intrinsic noise in *Bacillus subtilis*

promoter induction
via IPTG or ...



Induction of the *lac* Operon

Intrinsic noise in *Bacillus subtilis*

... or point mutations in promoter

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

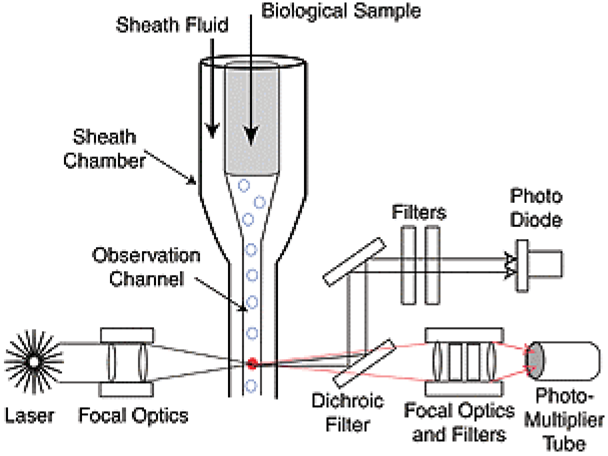
Strain	Ribosome binding site	Initiation codon	Translational efficiency
ERT25	GGG AAA AGG AGG TGA ACT ACT	ATG	1.00
ERT27	GGG AAA AGG AGG TGA ACT ACT	<u>T</u>TG	0.87
ERT3	GGG AAA AGG <u>T</u>GG TGA ACT ACT	ATG	0.84
ERT29	GGG AAA AGG AGG TGA ACT ACT	<u>G</u>TG	0.66

point mutations in ribosomal binding site (RBS) and initiation codon of *gfp*

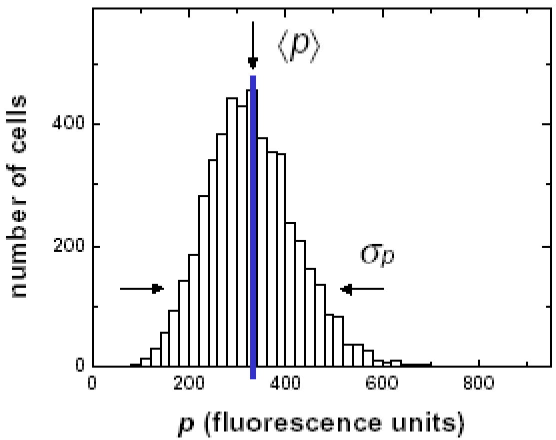
Table 2 • Transcriptional mutants: point mutations in the P_{spac} promoter

Strain	−10 regulatory region −10 +1	Transcriptional efficiency
ERT57	CAT AAT GTG TG<u>T</u> AAT	6.63
ERT25	CAT AAT GTG TGG AAT	1.00
ERT53	CAT AAT GTG TG<u>C</u> AAT	0.79
ERT51	CAT AAT GTG TG<u>A</u> AAT	0.76
ERT55	CAT AAT GTG T<u>AA</u> AAT	0.76

Intrinsic noise in *Bacillus subtilis*



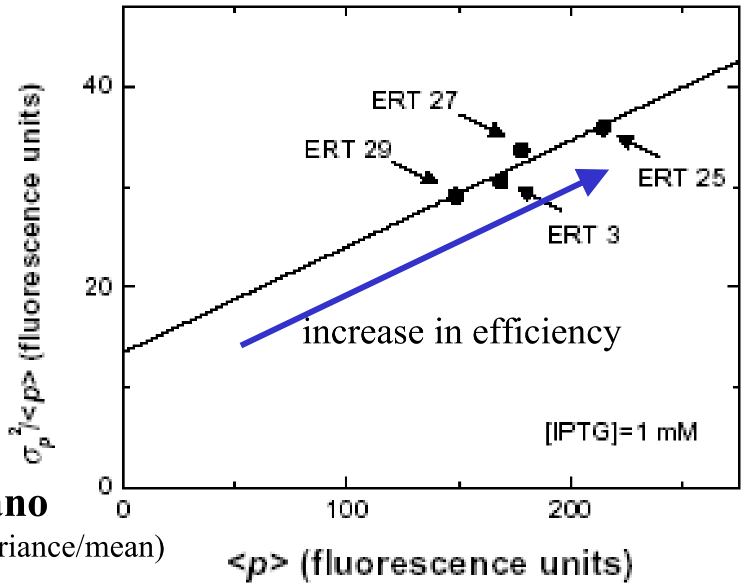
GFP expression level is measured for single cells in a bacterial population using flow cytometry



Expression level vary from cell to cell (phenotypic noise) as a consequence of molecular fluctuations within single cells (biochemical noise)

Intrinsic noise in *Bacillus subtilis*

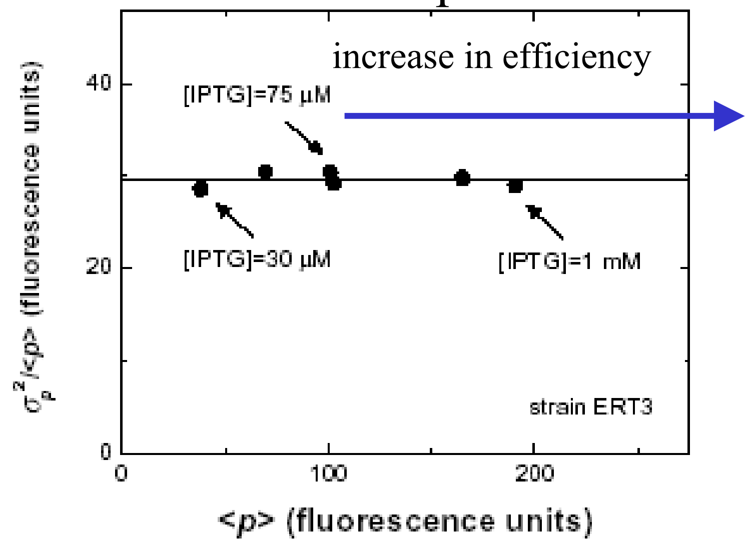
translation



Fano
(variance/mean)

translational efficiency
vs.
transcriptional efficiency

transcription



Intrinsic noise in *Bacillus subtilis*

Recall: gene expression model

$$\frac{d[mRNA]}{dt} = k_R - \delta_R[mRNA]$$
$$\frac{d[P]}{dt} = k_P[mRNA] - \delta_P[P]$$

$$\begin{aligned} \emptyset &\xrightarrow{k_R} mRNA, \\ mRNA &\xrightarrow{\delta_R} \emptyset, \\ mRNA &\xrightarrow{k_P} P, \\ P &\xrightarrow{\delta_P} \emptyset, \end{aligned}$$

Master equation valid to ...

$$\begin{aligned} \frac{dp_{m,n}}{dt} &= -p_{m,n}[m\delta_R + mk_P + k_R + n\delta_P] \\ &+ p_{m,n+1}(n+1)\delta_P + p_{m+1,n}(m+1)\delta_R \\ &+ p_{m,n-1}k_P m + p_{m-1,n}k_R \end{aligned}$$

Intrinsic noise in *Bacillus subtilis*

... to get the final expressions for the macroscopic statistics

$$\text{Fano Protein} = \frac{\langle n^2 \rangle - \langle n \rangle^2}{\langle n \rangle} = 1 + \frac{k_P/\delta_R}{1+\delta_P/\delta_R} \approx \boxed{1 + \frac{k_P}{\delta_R}}$$

translation efficiency influences noise

$$\text{Fano mRNA} = \boxed{1}$$

protein half-lifetime ~ hours
mRNA half-lifetime ~ minutes
thus

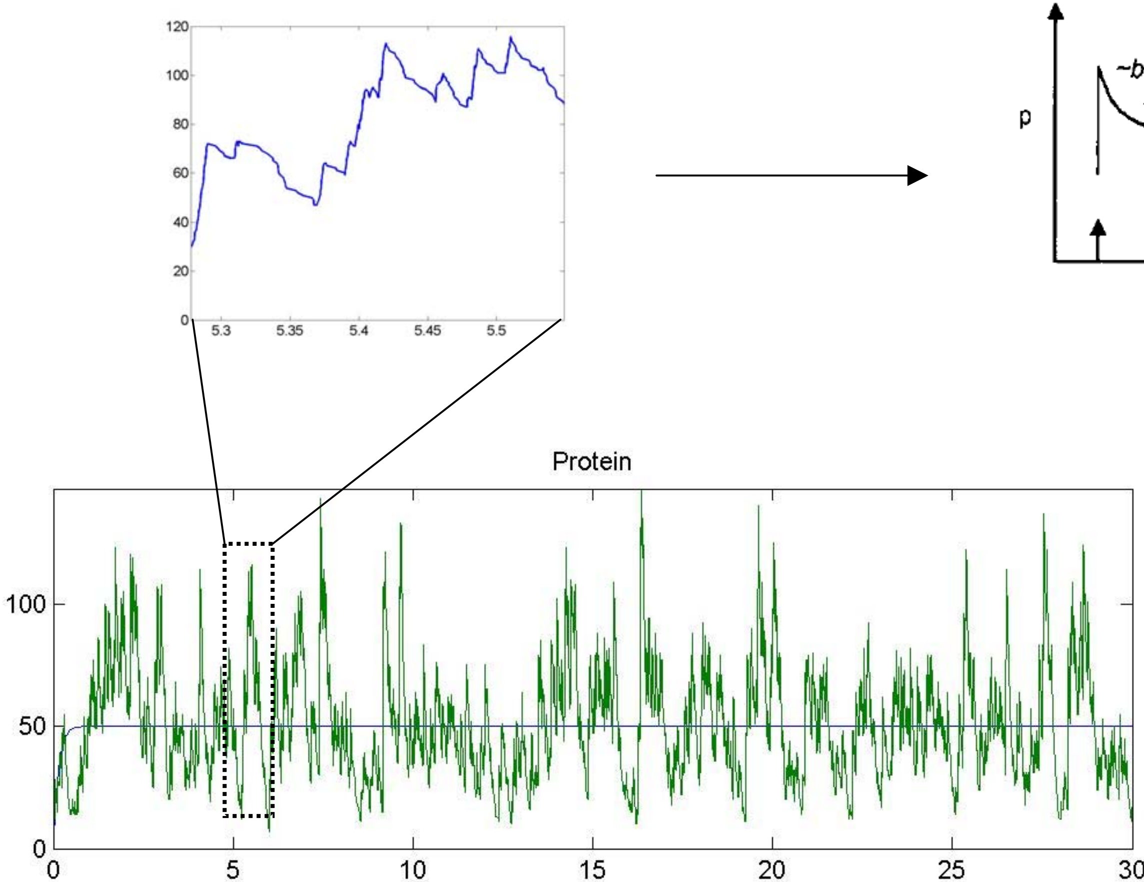
$t_{1/2} = \log 2/\delta$ and $\delta_P \ll \delta_R$

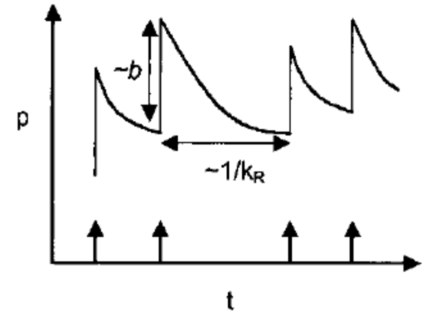
transcription efficiency does not influence noise

Intrinsic noise in *Bacillus subtilis*

"Random bursts model"

$$b = \frac{k_P}{\delta_R}$$





Translational noise control

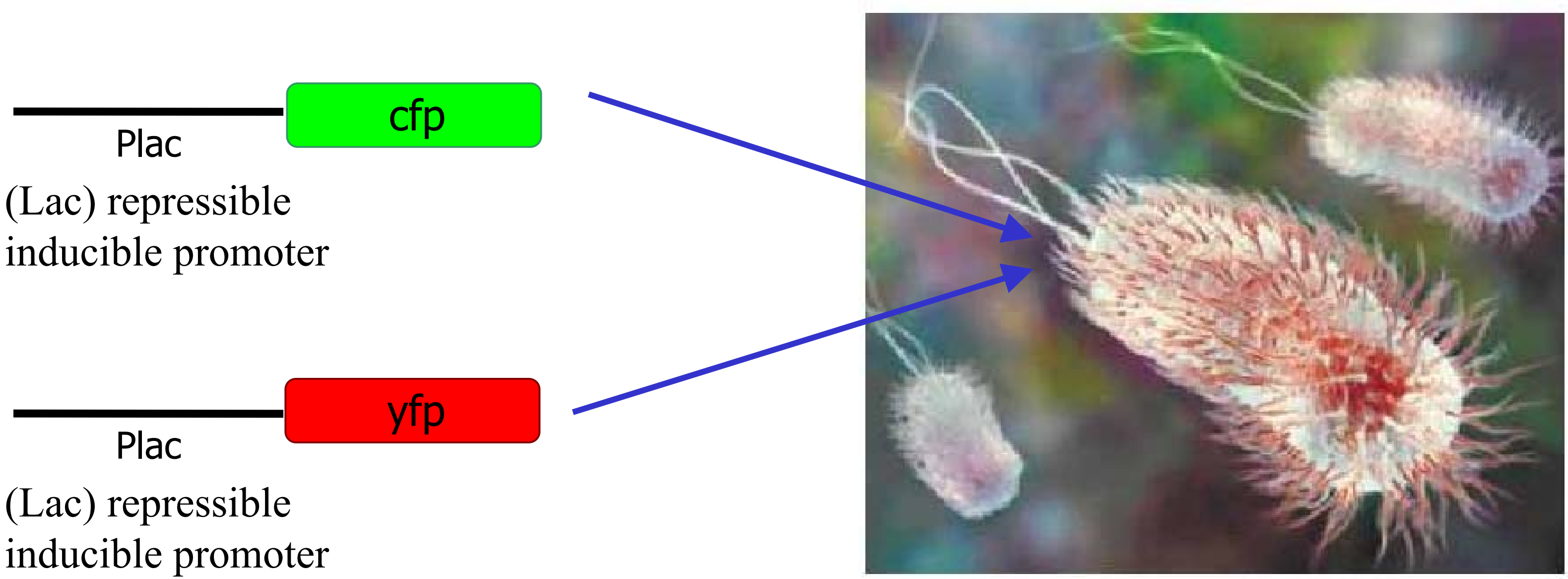
Intrinsic/Extrinsic noise in *Escherichia coli*

Intrinsic noise, even if all cellular conditions are equivalent for cells, we have seen that the reactions associated to transcription and translation originate noise

Extrinsic noise, other molecular species (genes themselves too!), e.g., RNA polymerase, originate noise

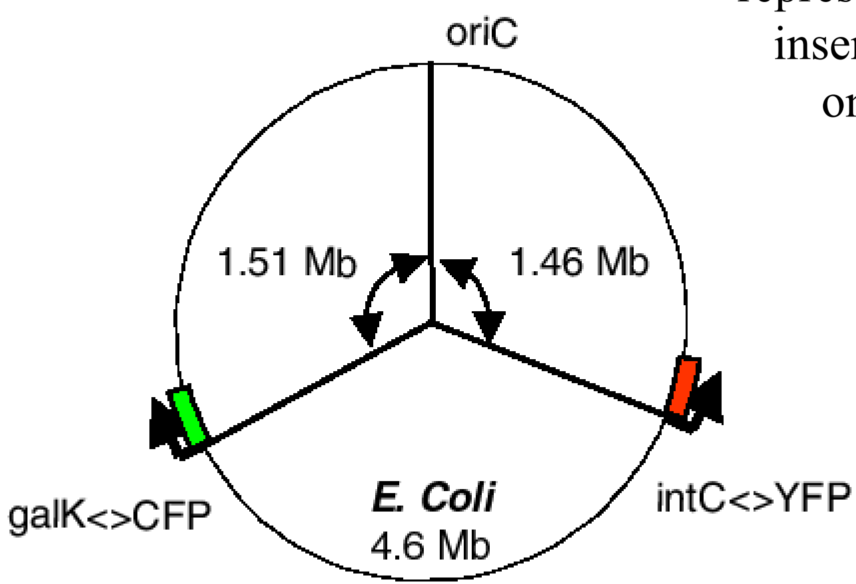
Can we discriminate both sources of noise?

Intrinsic noise:= Difference in gene expression that arises between two identical copies of a gene expressed under precisely the same conditions



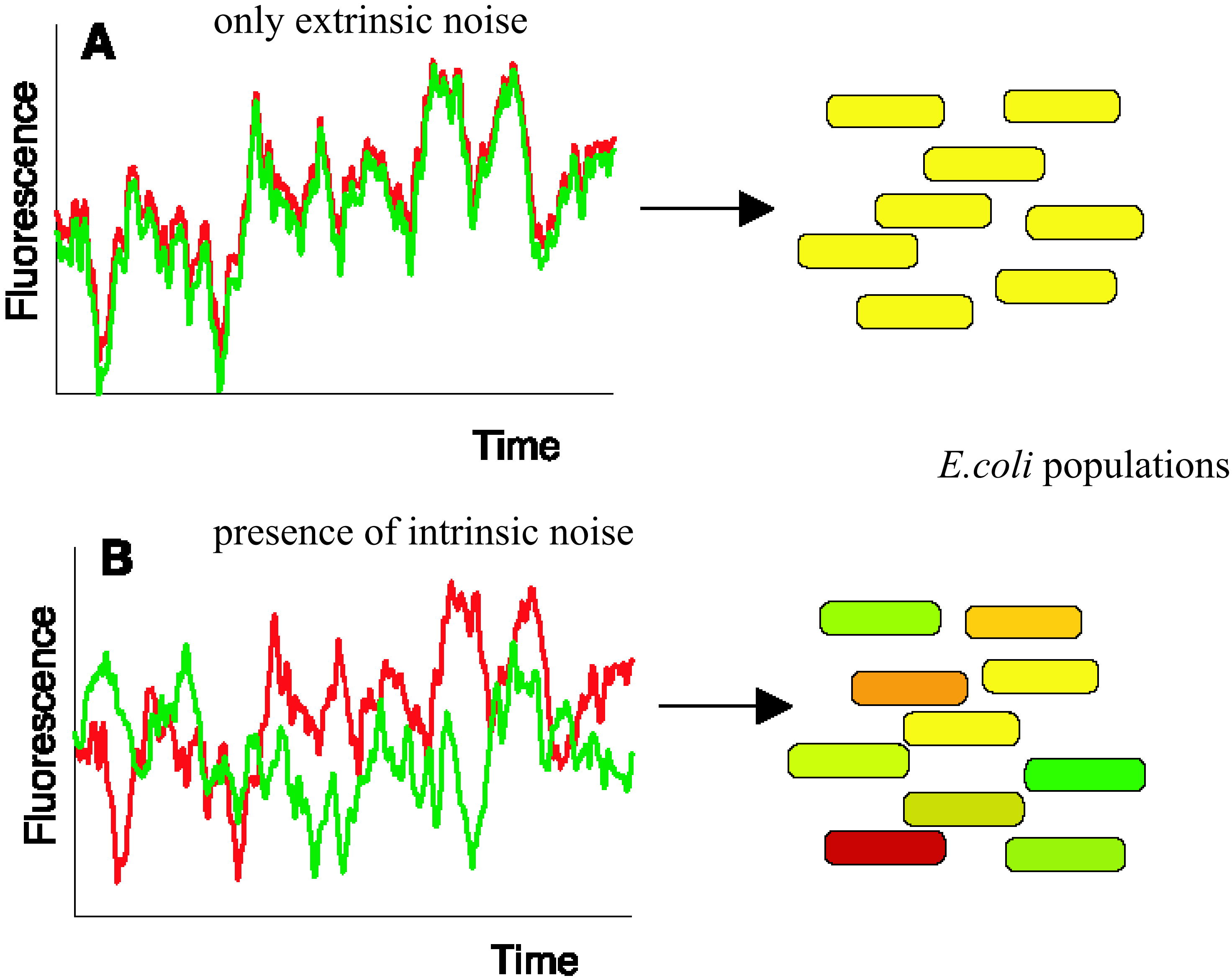
Intrinsic/Extrinsic noise in *Escherichia coli*

Intrinsic noise:= Difference in gene expression that arises between two identical copies of a gene expressed under precisely the same conditions

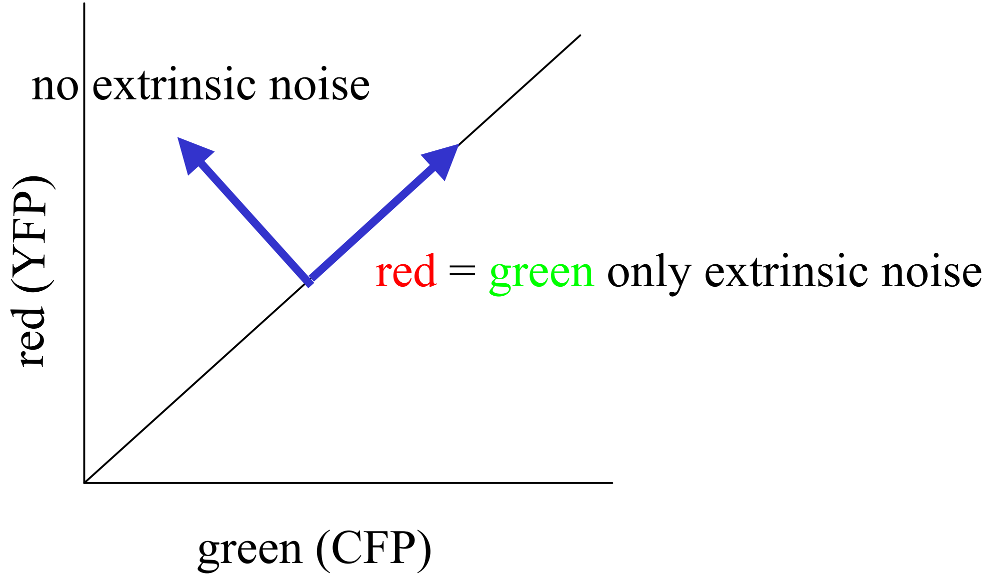


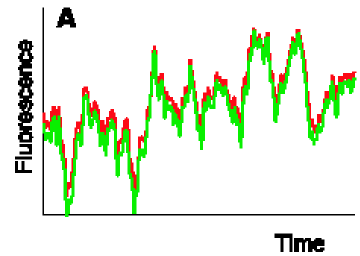
Two virtually equivalent Lac-repressible GFP reporter genes inserted in the *E.coli* chromosome on opposite sites and roughly equivalent to the origin of replication

Intrinsic/Extrinsic noise in *Escherichia coli*



$$\xi^2_{\text{total}} = \xi^2_{\text{intrinsic}} + \xi^2_{\text{extrinsic}} \; ?$$





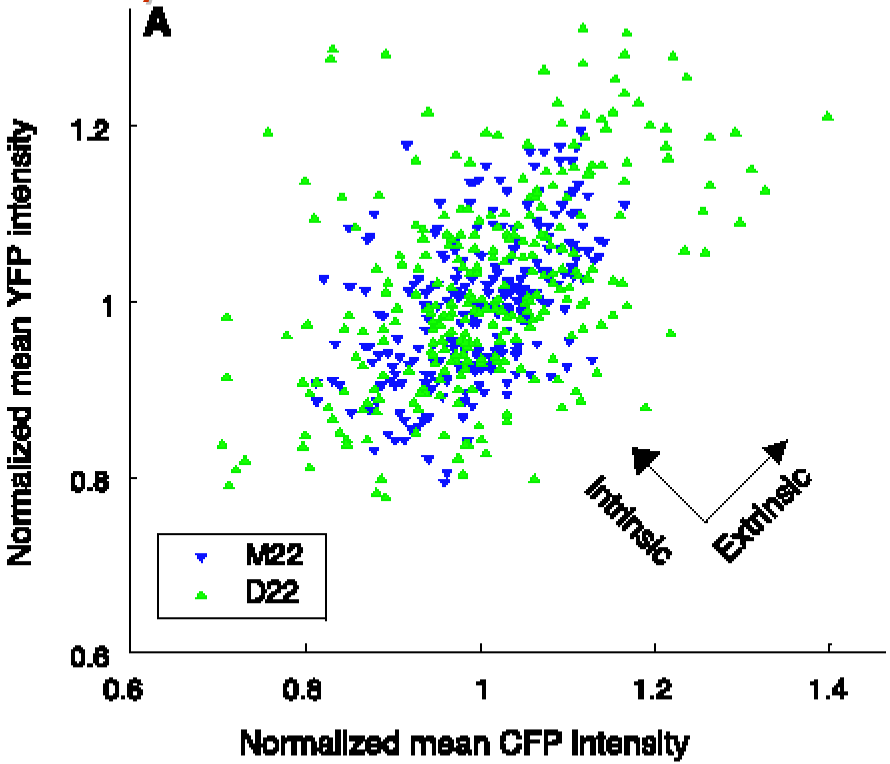
since $$\frac{1}{N}\sum_{k=1}^{N} P_k^m \approx \int d\mathbf{E}\, d\mathbf{I}\, P^m(\mathbf{E},\mathbf{I})\, p(\mathbf{E}\mathbf{I}) = \int d\mathbf{E}\, p(\mathbf{E}) \int d\mathbf{I}\, P^m(\mathbf{E},\mathbf{I})\, p(\mathbf{I}|\mathbf{E})$$

$$= \int d\mathbf{E}\, p(\mathbf{E}) \langle P^m(\mathbf{E}) \rangle = \overline{\langle P^m \rangle}$$

then $$\xi^2_{\text{total}} = \frac{\overline{\langle P^2 \rangle} - (\overline{\langle P \rangle})^2}{(\overline{\langle P \rangle})^2} = \frac{\overline{\langle P^2 \rangle - \langle P \rangle^2}}{(\overline{\langle P \rangle})^2} + \frac{\overline{\langle P \rangle^2} - (\overline{\langle P \rangle})^2}{(\overline{\langle P \rangle})^2}$$

$$\equiv \xi^2_{\text{int}} + \xi^2_{\text{ext}}$$

Intrinsic/Extrinsic noise in *Escherichia coli*



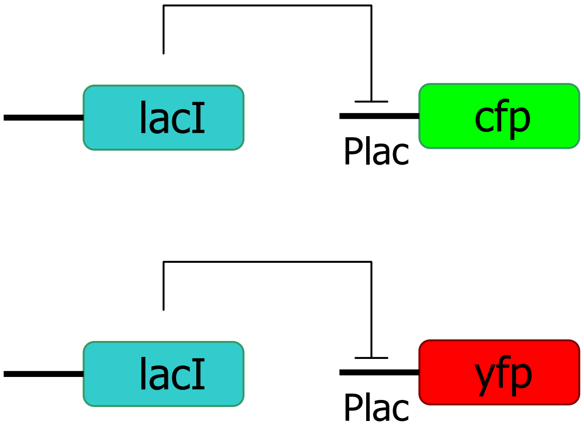
$$\text{noise} = \frac{\text{variance } (\sigma^2)}{\text{mean}^2}\ ; \qquad \boxed{\text{noise}^2_{\text{total}}\ (\xi) = \text{noise}^2_{\text{intrinsic}} + \text{noise}^2_{\text{extrinsic}}}$$

different to previous definition $n_2 = \dfrac{\sigma^2}{\langle n \rangle}$

Intrinsic/Extrinsic noise in *Escherichia coli*

(lacI$^-$ cells)

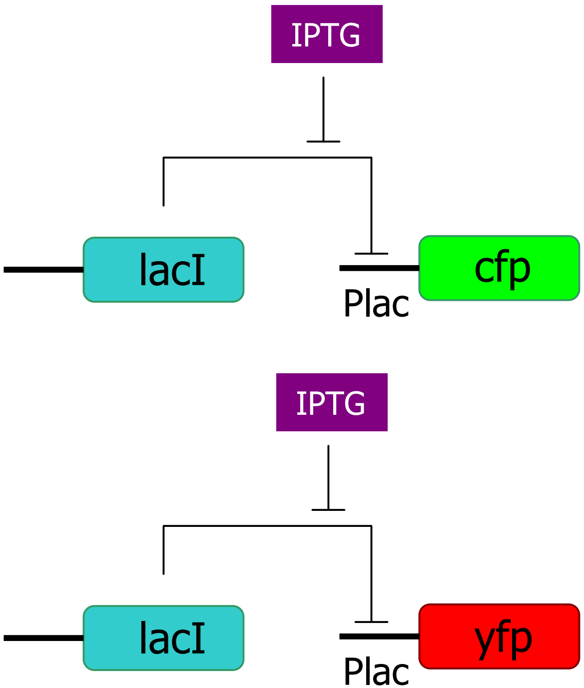
strong **constitutive promoter**
stable protein
high transcription low noise

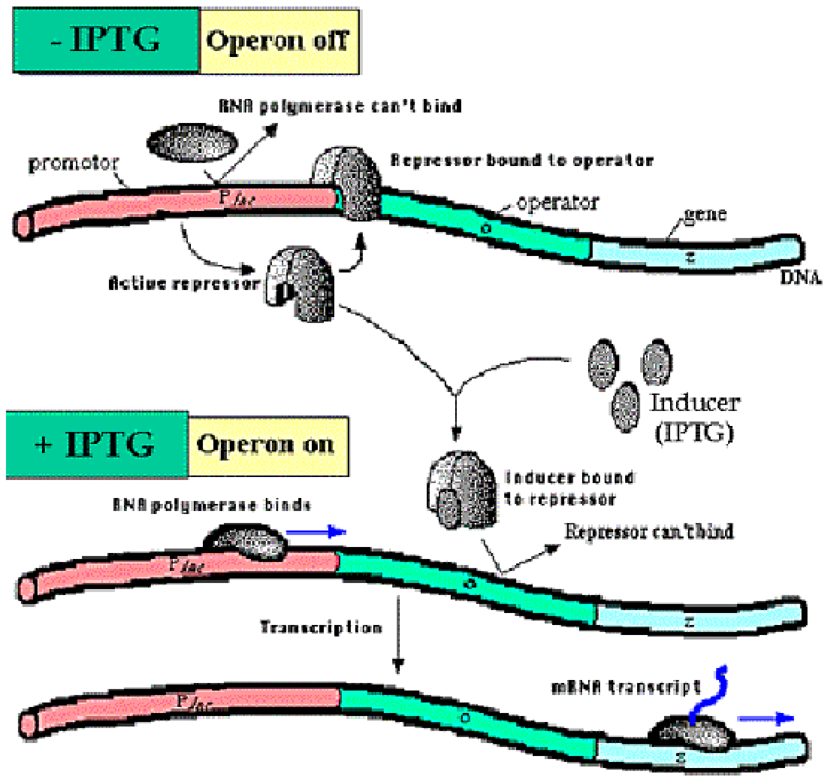


Intrinsic/Extrinsic noise in *Escherichia coli*

(lacI^{+} cells)

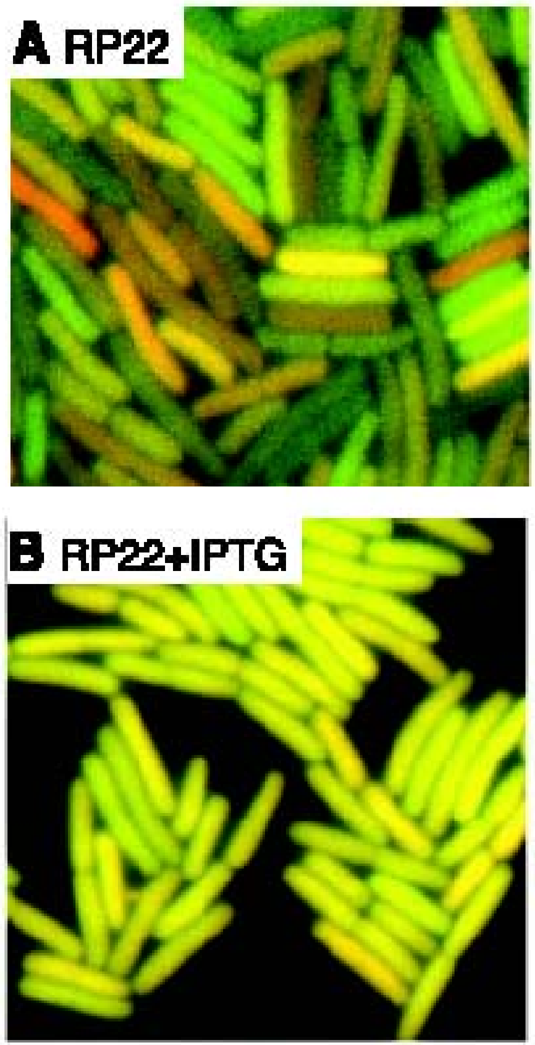
inducible promoter





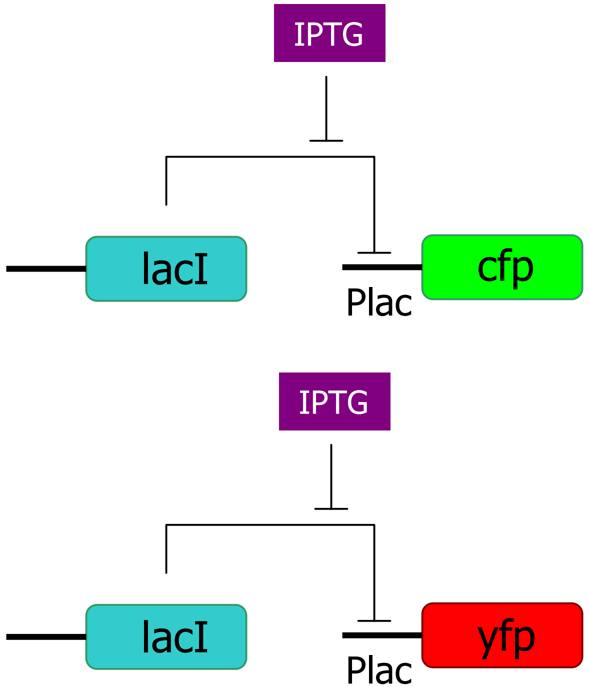
Induction of the *lac* Operon

Intrinsic/Extrinsic noise in *Escherichia coli*

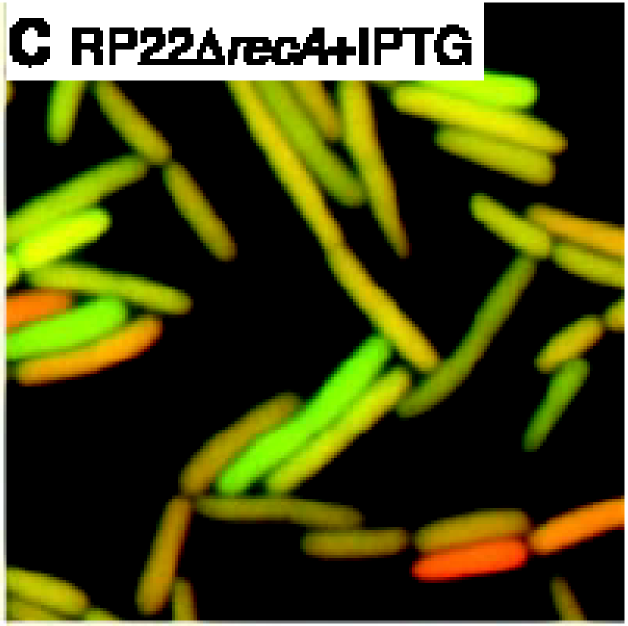


Promotors repressed by wild-type repressor (lacI) gene
(-IPTG operon OFF) low transcription, high noise

Presence of inducer (+IPTG operon ON)
high transcription, low noise

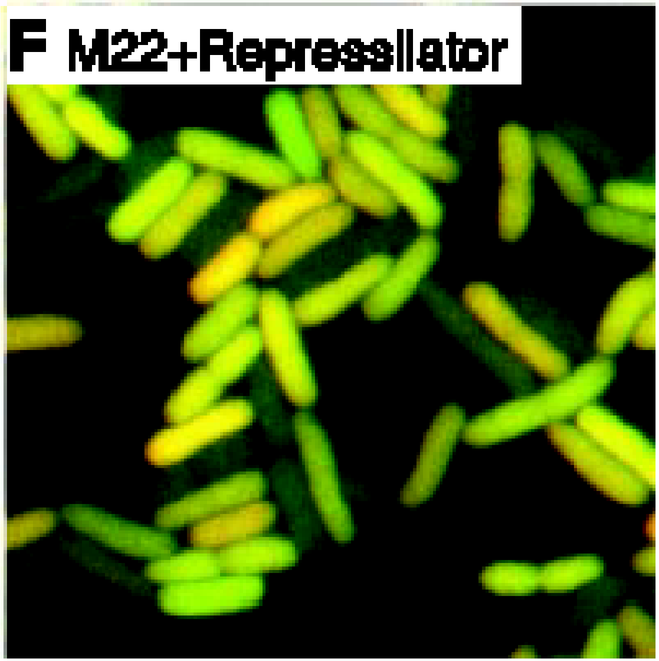


Intrinsic/Extrinsic noise in *Escherichia coli*

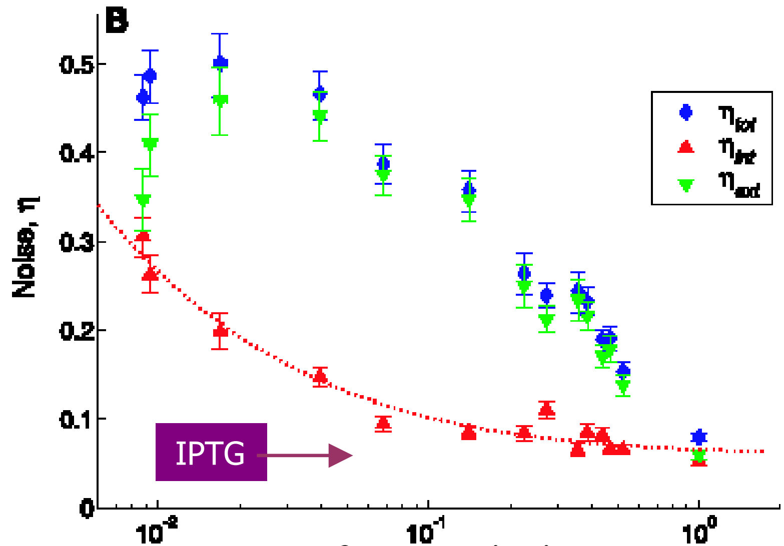


modified genetic
background
noisy

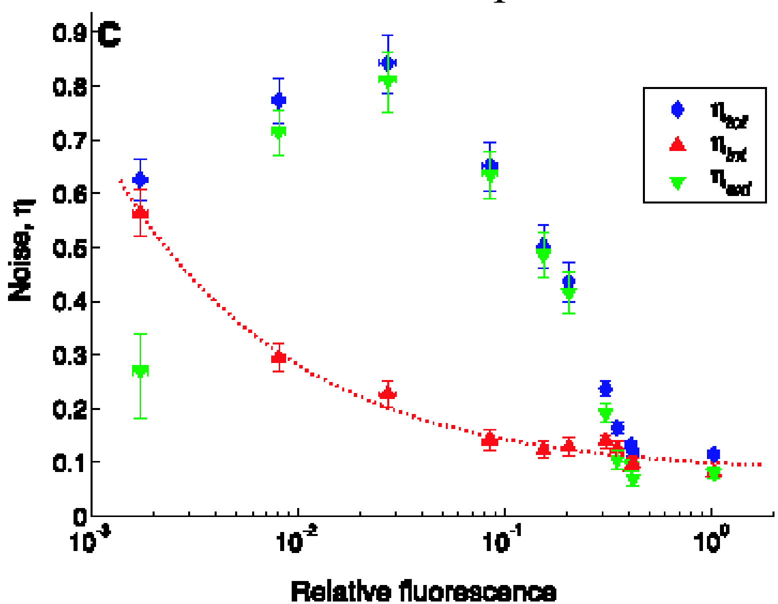
oscillating
expression
also noisy



Intrinsic/Extrinsic noise in *Escherichia coli*



rate of transcription



population means

(recA^{+} lacI^{-} cells)
LacI in plasmid

intrinsic noise decreases with rate of transcription (transcription in these experiments does have an effect on noise!)

extrinsic noise peaks at intermediate levels (fluctuations in Lac repressor proteins. At high or low IPTG concentrations fluctuations are buffered by excess IPTG or excess LacI, respectively)

Conclusions

- Phenotypic noise in a population as a consequence of protein concentration fluctuations.

-Translation and transcription leads to a control of fluctuations in protein concentration. Translation amplifies transcriptional noise.

- Some genes might have been naturally selected to have inefficient translational rates (a small rate of proteins per transcript) to avoid these fluctuations and thus avoid noise.

- In some circumstances noise can be highly desiderable as a means of creating nongenetic individuality in a population. In some other circumstances noise must be reduced (by means for instance of redundacy or negative feedback).

-Intrinsic and extrinsic sources of noise can be discriminated and measured.

-Theory + experiments + simulations a valid combined tool for biological discovery !!