# Biology of the *noisy* gene

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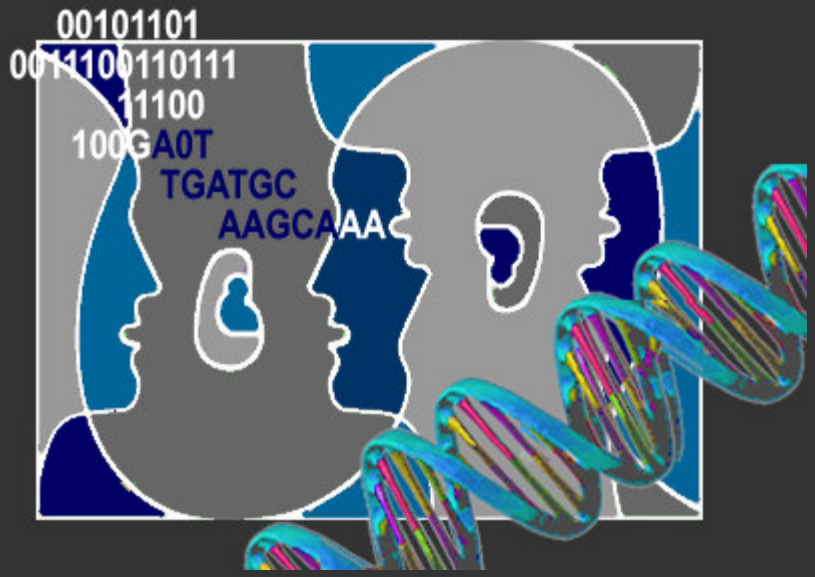
# day III: noisy bacteria




- Regulation of noise (*B. subtilis*)
- Intrinsic/Extrinsic noise (*E. coli*)
- Noise time scales

Logs laboratory

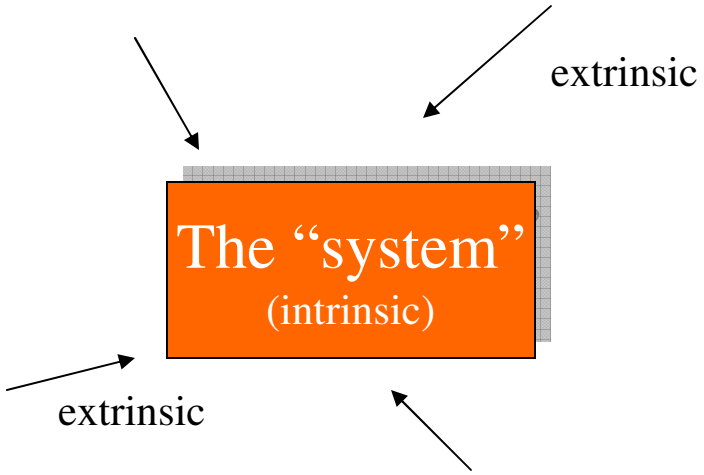
# Stochastic dynamics of gene expression, experiments!

- Intrinsic noise in *Bacillus subtilis*

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

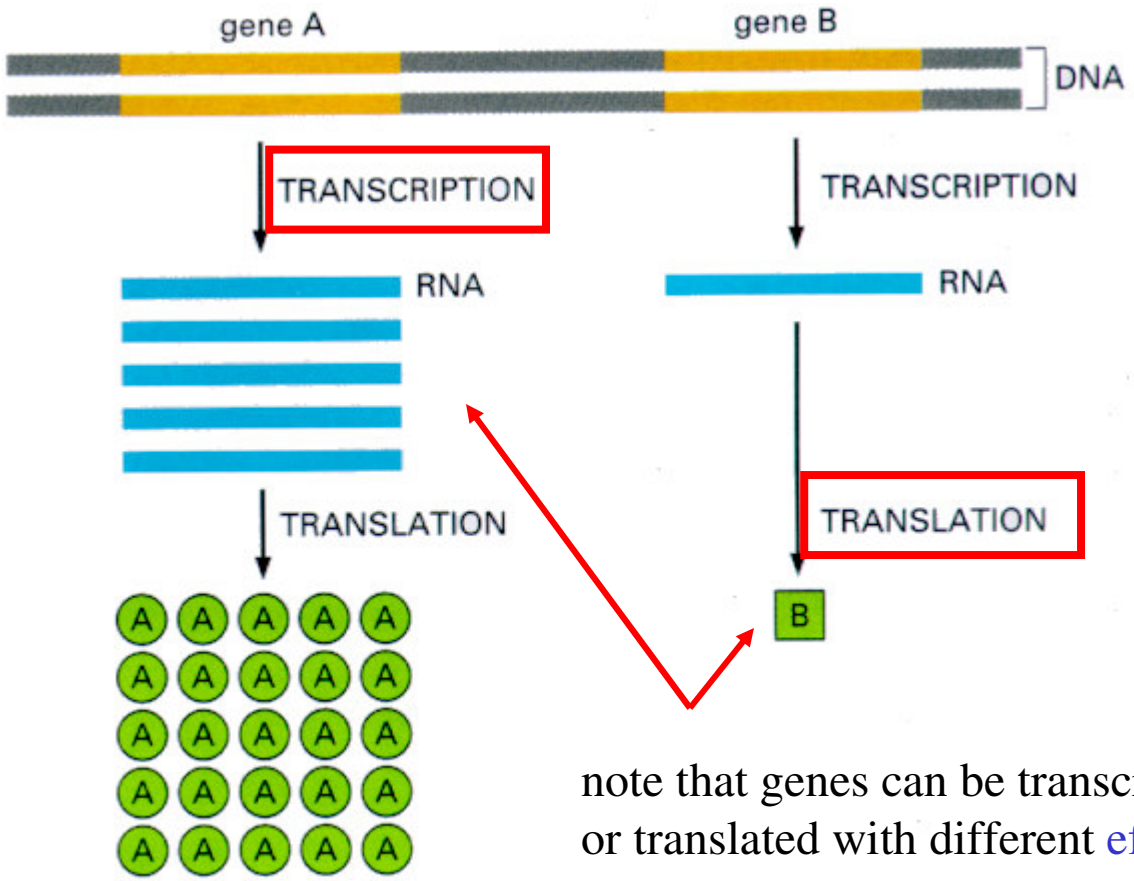
- Extrinsic *vs.* intrinsic noise in *Escherichia coli*

Detection of noise and discrimination between intrinsic and extrinsic noise



# Intrinsic noise in *Bacillus subtilis*

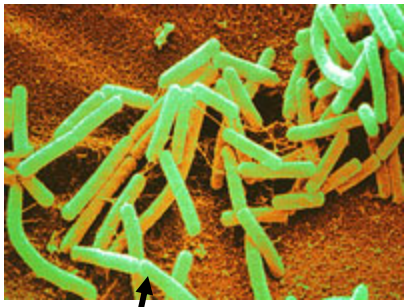
Gene expression in a nutshell

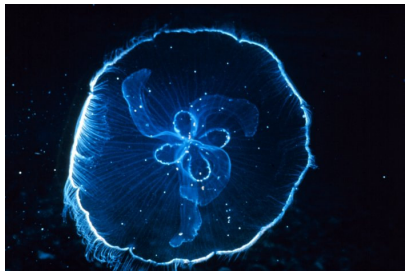


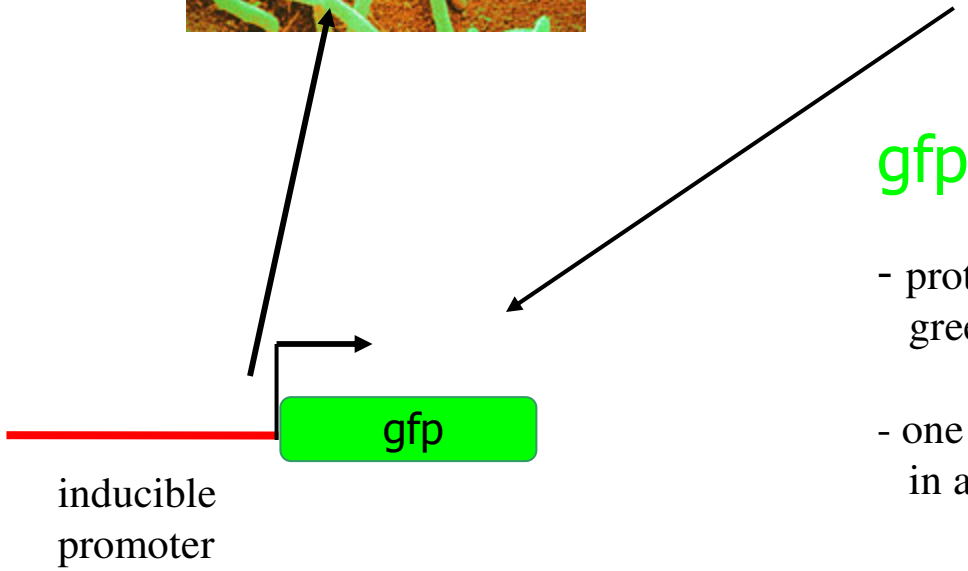

note that genes can be transcripted
or translated with different efficiencies/rates

# Intrinsic noise in *Bacillus subtilis*

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








gfp

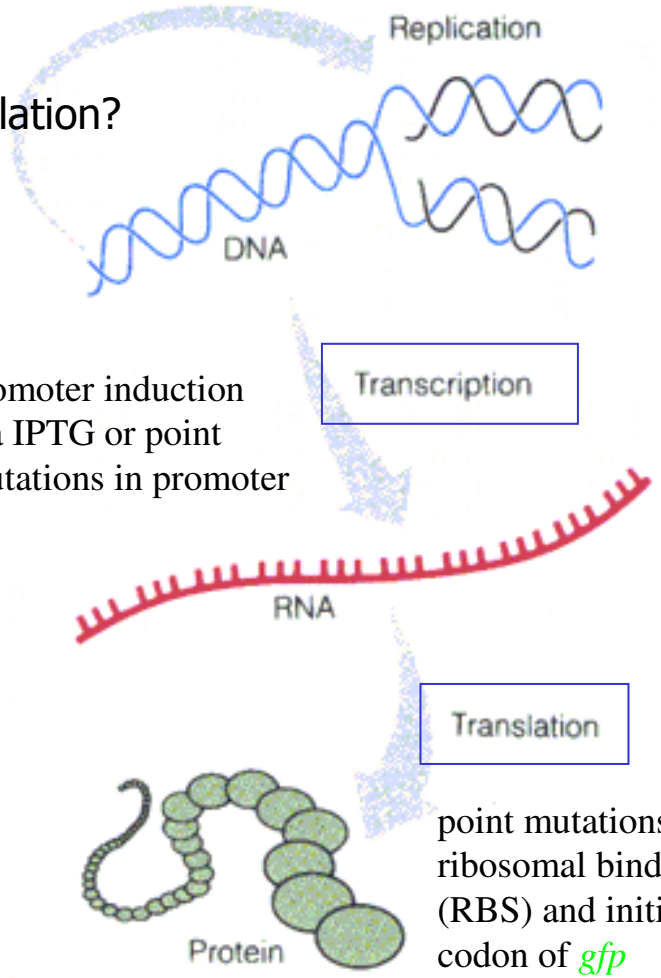
- protein from **jellyfish** that fluoresces green when exposed to light.

- 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?



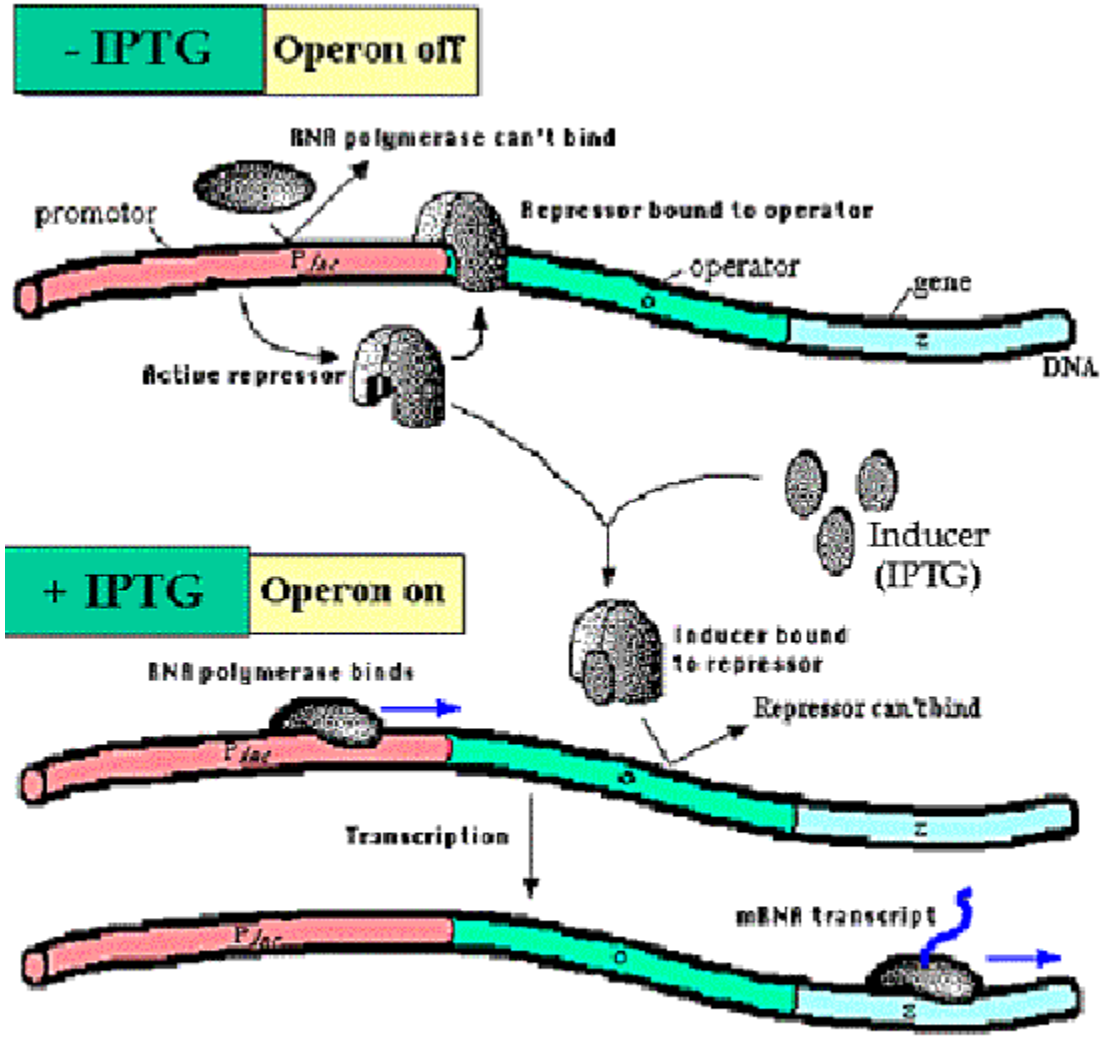

promoter induction
via IPTG or point
mutations in promoter

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

# Intrinsic noise in *Bacillus subtilis*

Transcription efficiency

promoter induction via IPTG or ...




Induction of the *lac* Operon

# Intrinsic noise in *Bacillus subtilis*

## Transcription efficiency

... or point mutations in promoter

**• Transcriptional mutants: point mutations in the $P_{spac}$ promoter**

| Strain | −10 regulatory region<br>−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 |

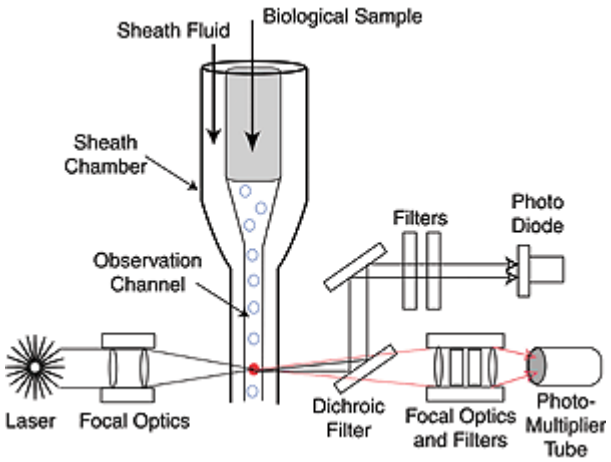
## Translational efficiency

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

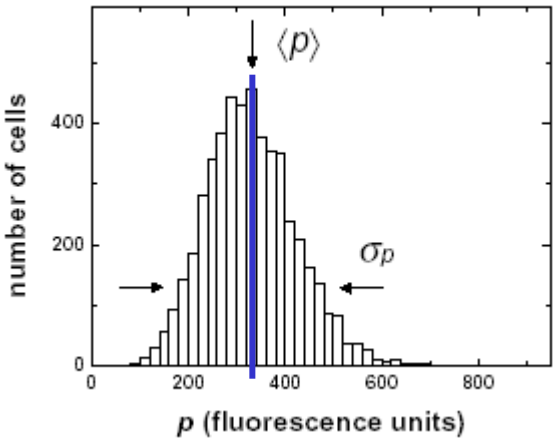
**• Translational mutants: point mutations in the RBS and initiation codon of *gfp***

| 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 |

# Intrinsic noise in *Bacillus subtilis*



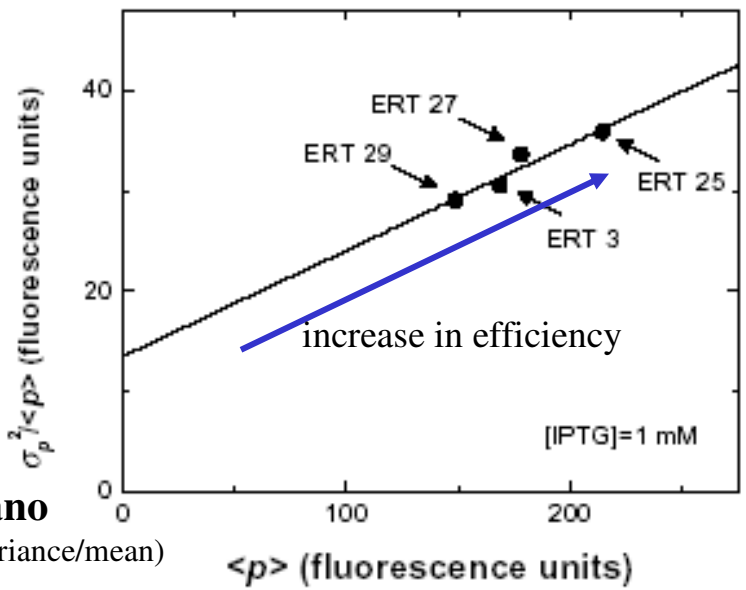

GFP expression level is measured for single cells in a bacterial population using flow cytometry




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*

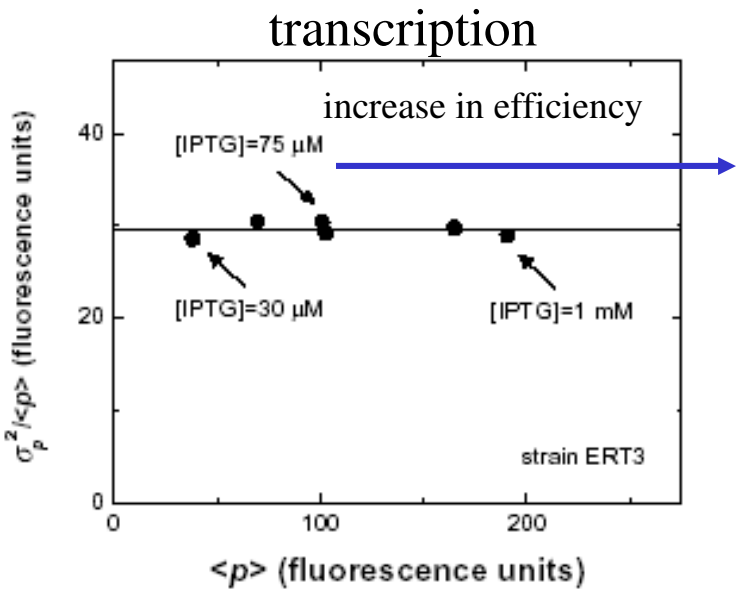
translation




**Fano**
(variance/mean)

translational efficiency
vs.
transcriptional efficiency

transcription



# Intrinsic noise in *Bacillus subtilis*

Recall: gene expression model

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

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

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

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

translation efficiency influences noise

Fano mRNA = $\boxed{1}$

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

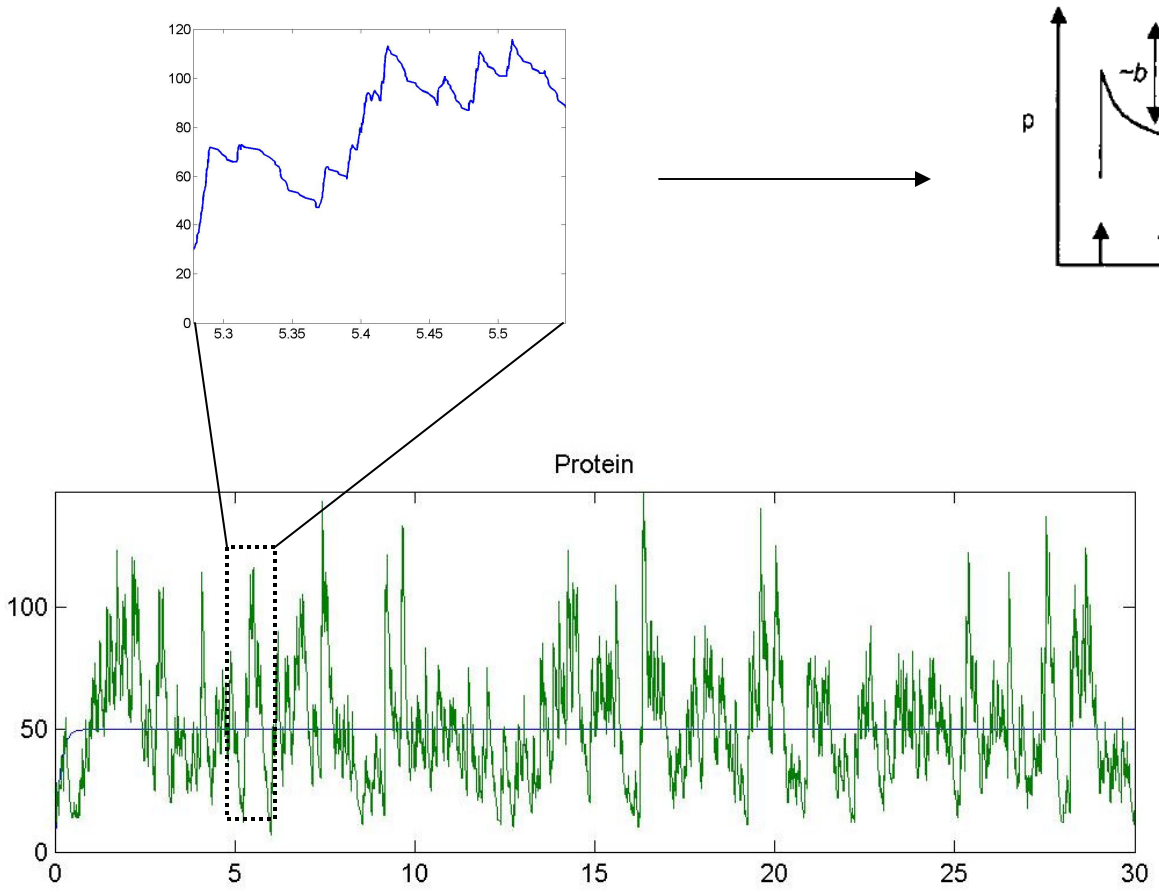
$t_{1/2} = \log 2/\delta$ and $\delta_P \ll \delta_R$

transcription efficiency does not influence noise

# Intrinsic noise in *Bacillus subtilis*

"Random bursts model"

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




Translational noise control

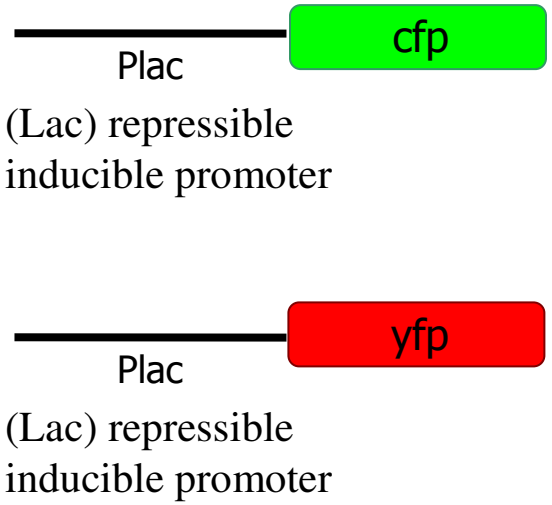
# Intrinsic/Extrinsic noise in *Escherichia coli*

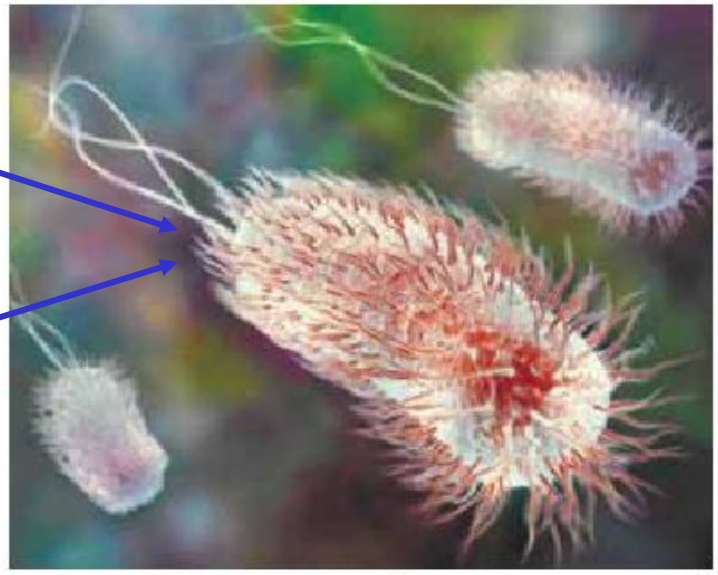
Intrinsic noise, even if all cellular conditions are equivalent for cells, we have seen that the reactions associated to transcription and translation originate noise

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



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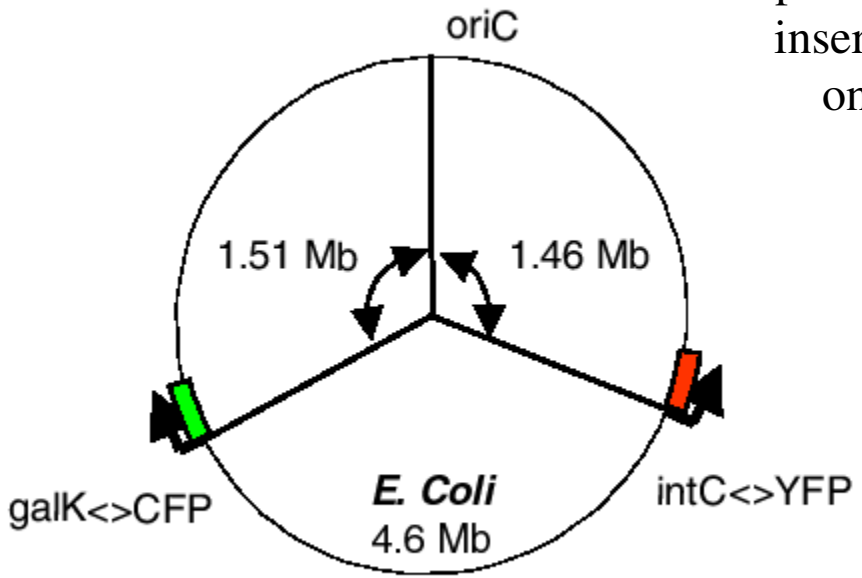
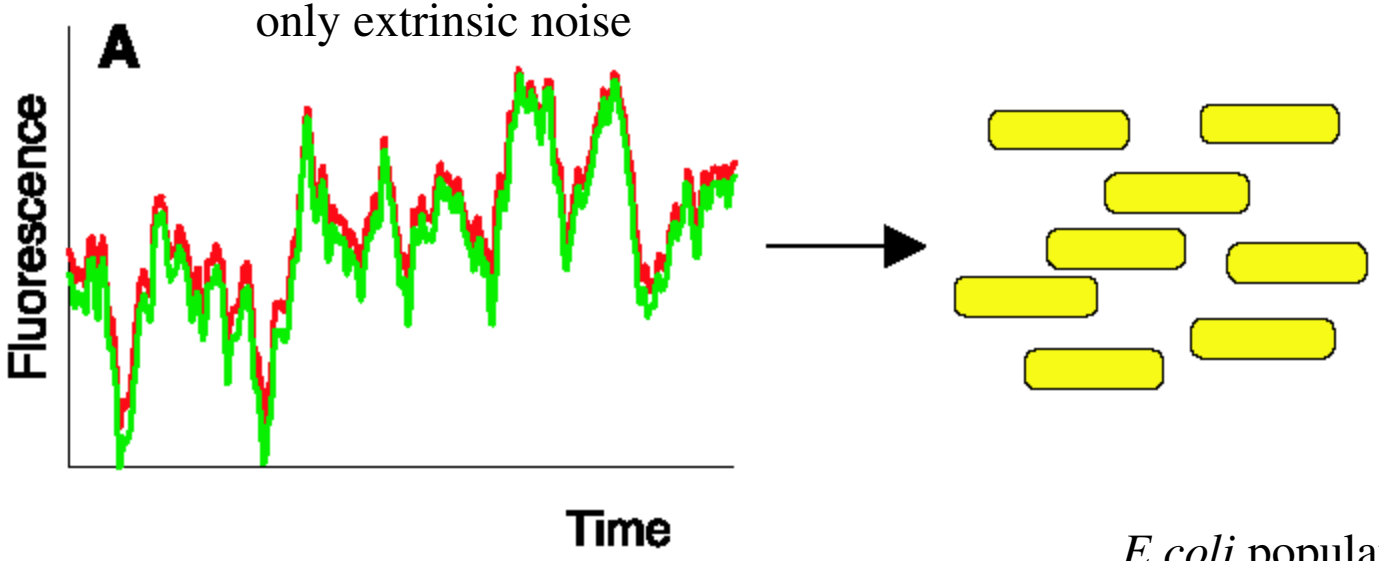



Two virtually equivalent Lac-repressible GFP reporter genes inserted in the *E.coli* chromosome on opposite sites and roughly equivalent to the origin of replication

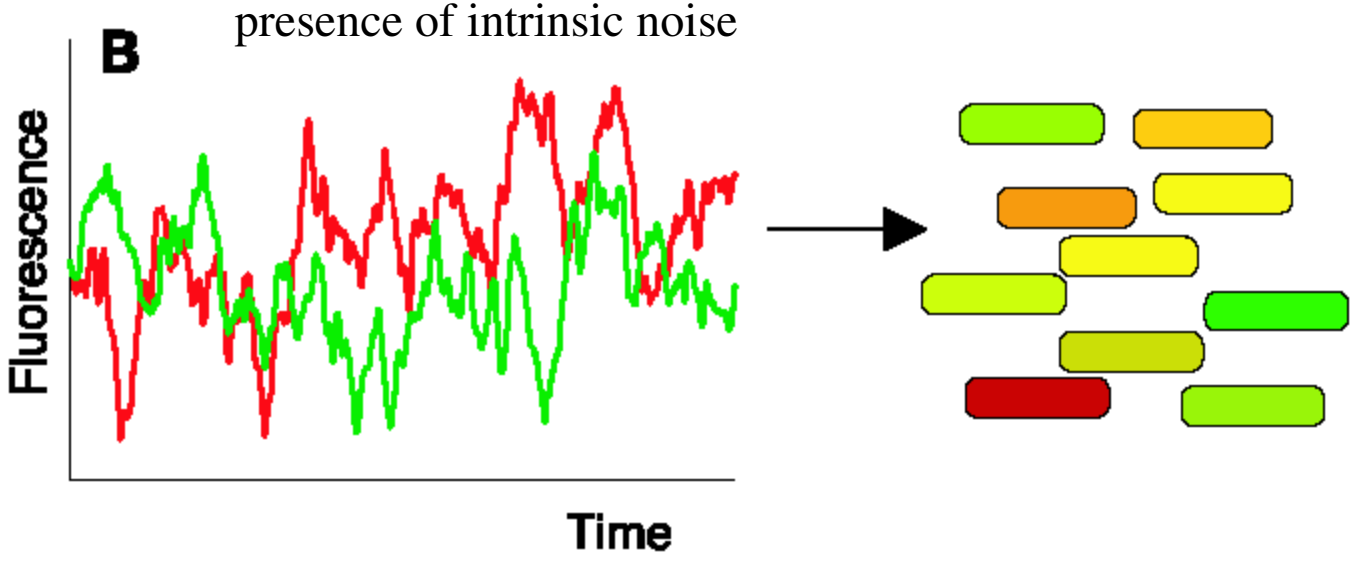
# Intrinsic/Extrinsic noise in *Escherichia coli*

only extrinsic noise




*E.coli* populations

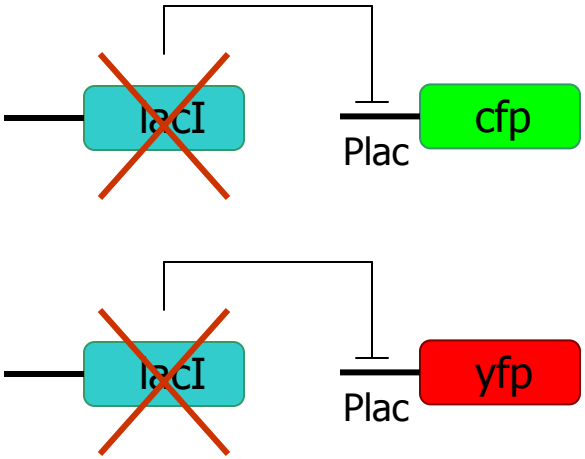
presence of intrinsic noise



# Intrinsic/Extrinsic noise in *Escherichia coli*

(lacI$^-$ cells)

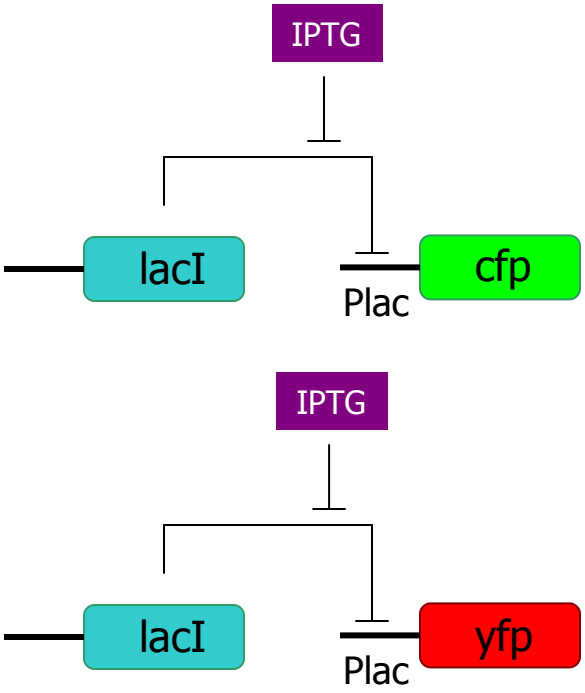
strong **constitutive promoter**
stable protein
high transcription low noise

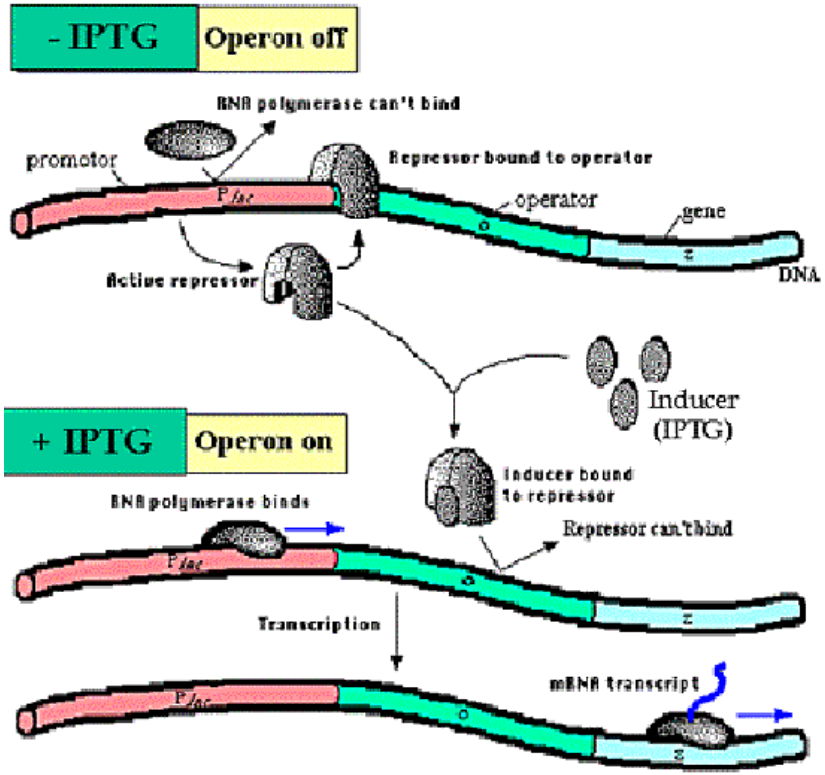



# Intrinsic/Extrinsic noise in *Escherichia coli*

(lacI$^+$ cells)

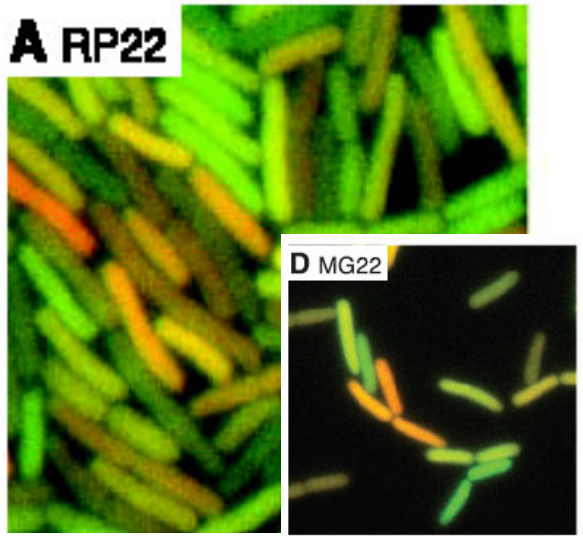
**inducible promoter**

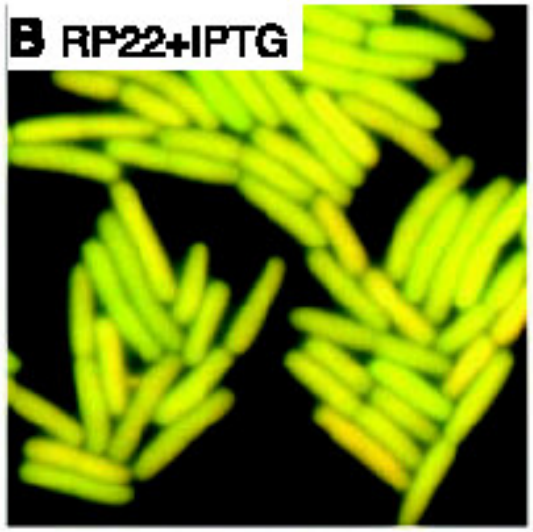



Induction of the *lac* Operon

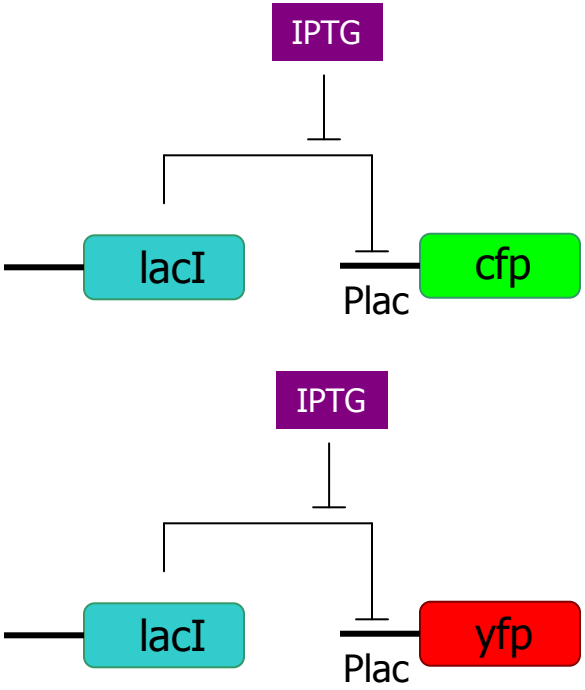
# Intrinsic/Extrinsic noise in *Escherichia coli*



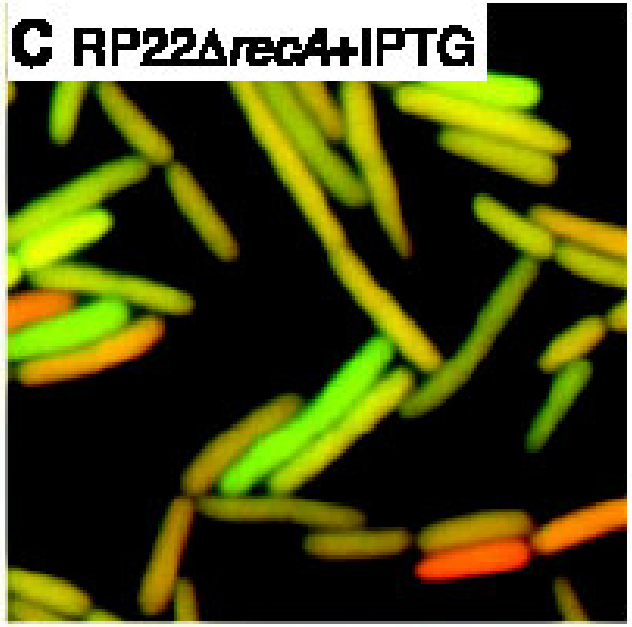




Promotors repressed by wild-type repressor (lacI) gene (-IPTG operon OFF) low transcription, high noise

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

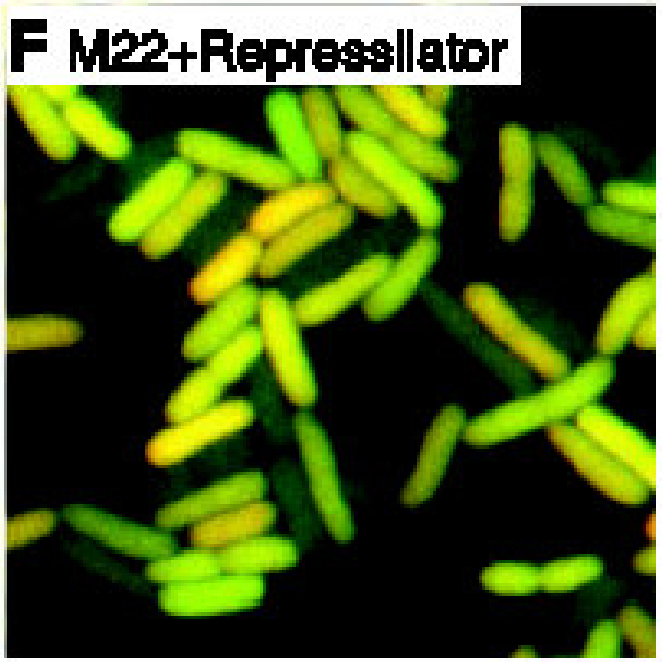


# Intrinsic/Extrinsic noise in *Escherichia coli*

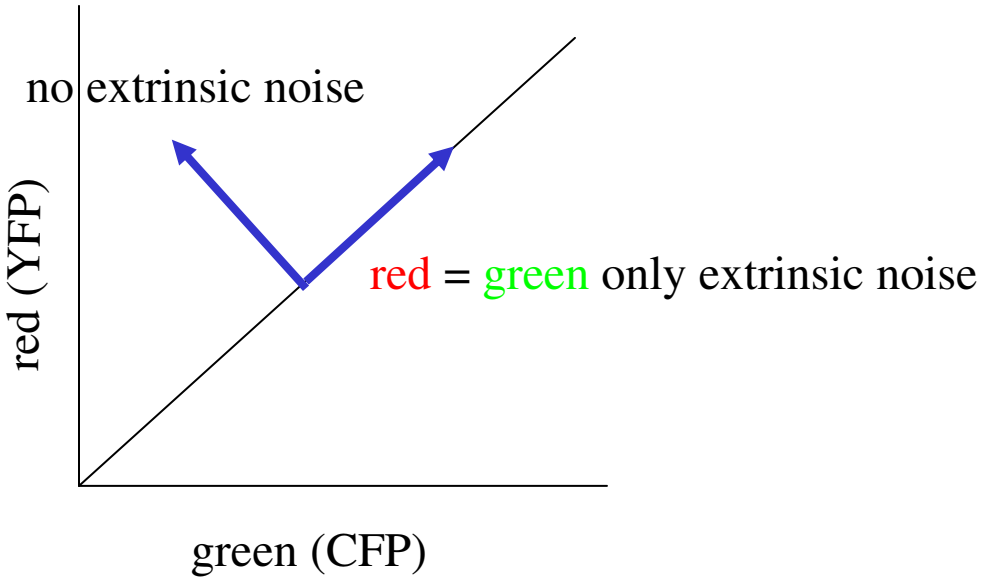


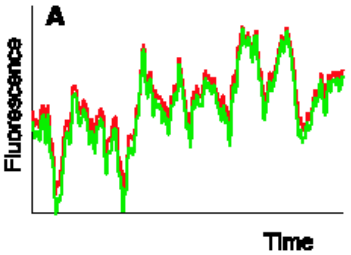

modified genetic
background
noisy

oscillating
expression
also noisy



# Intrinsic/Extrinsic noise in *Escherichia coli*



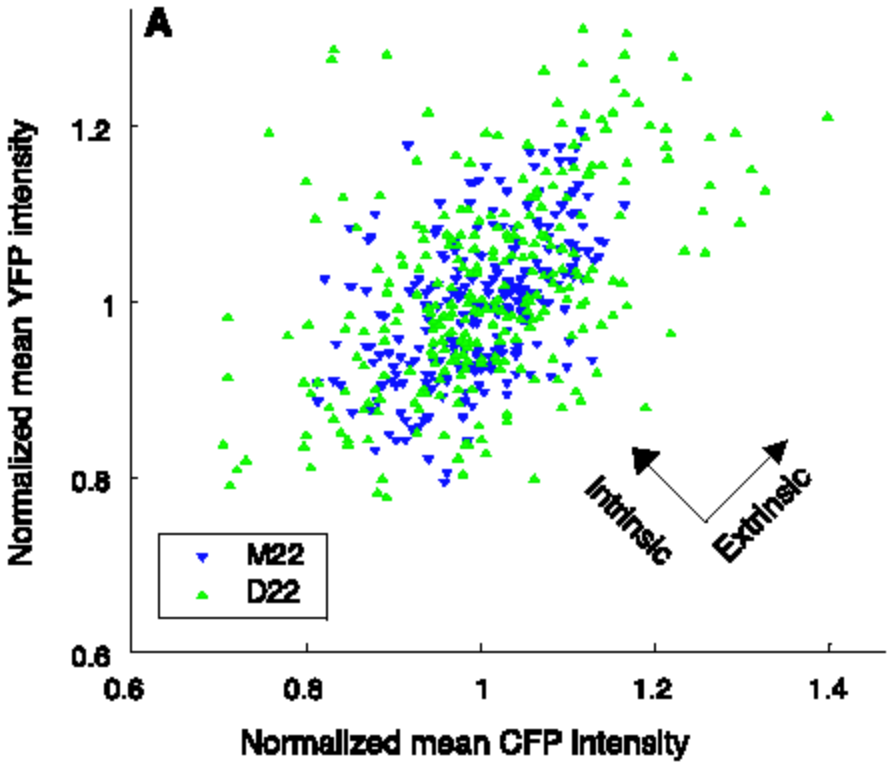




$$\text{noise} = \frac{\text{variance } (\sigma^2)}{\text{mean}^2} \; ;$$

$$\text{noise}^2_{\text{total}}\,(\xi) = \text{noise}^2_{\text{intrinsic}} + \text{noise}^2_{\text{extrinsic}}$$

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

# Intrinsic/Extrinsic noise in *Escherichia coli*



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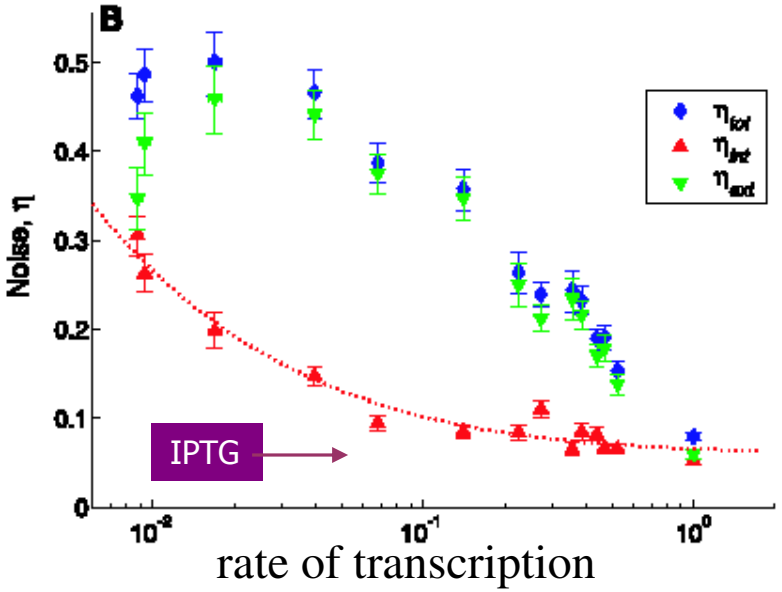


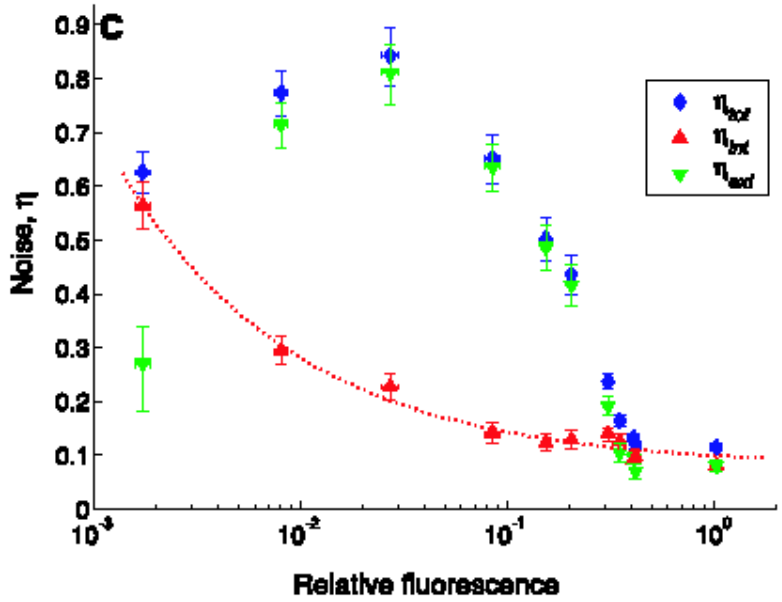


population means

(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)

# Intrinsic/Extrinsic noise; time scales