1. Why stochastic?

2. Mathematical descriptions

    (i) the master equation
    (ii) Langevin theory

3. Single cell measurements

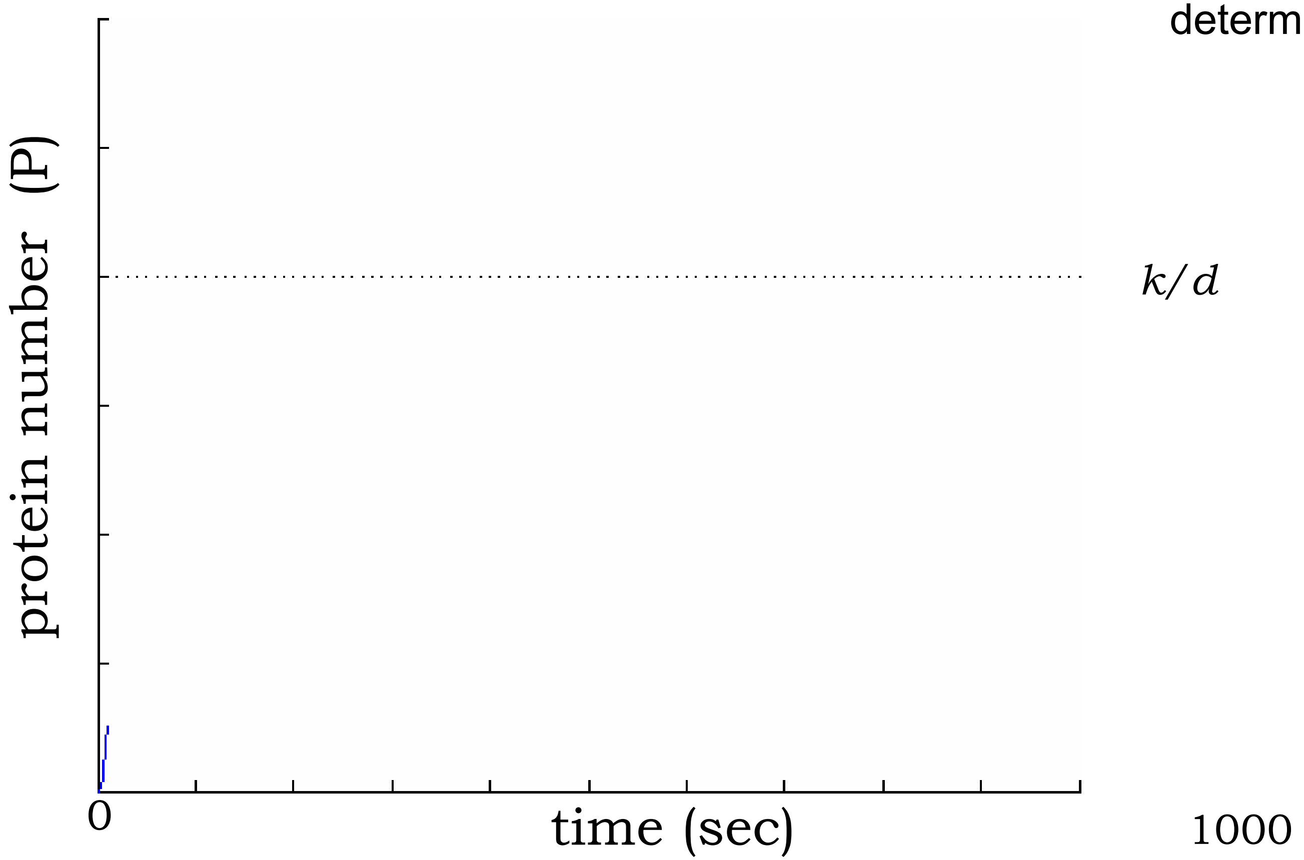
4. Consequences

Any chemical reaction is stochastic.

$$\xrightarrow{k} P \xrightarrow{d} \phi$$

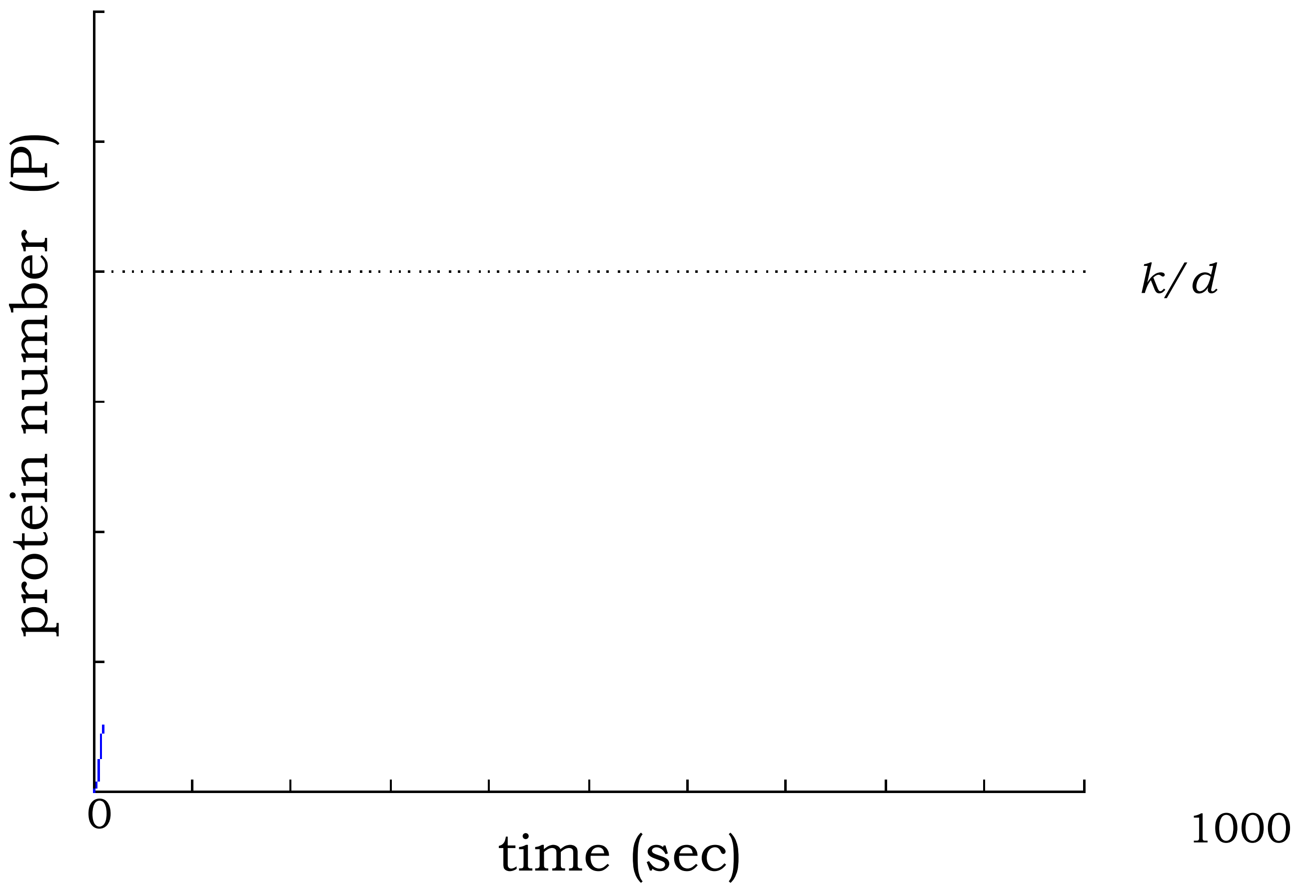
$$\frac{dP}{dt} = k - d \times P$$

deterministic equation



Any chemical reaction is stochastic.

$$\xrightarrow{k} P \xrightarrow{d} \phi$$



Why are chemical reactions stochastic?

$$A + B \xrightarrow{f} C$$

1. Reactants diffuse to find each other in solution.
2. They must overcome the energy barrier of the reaction.

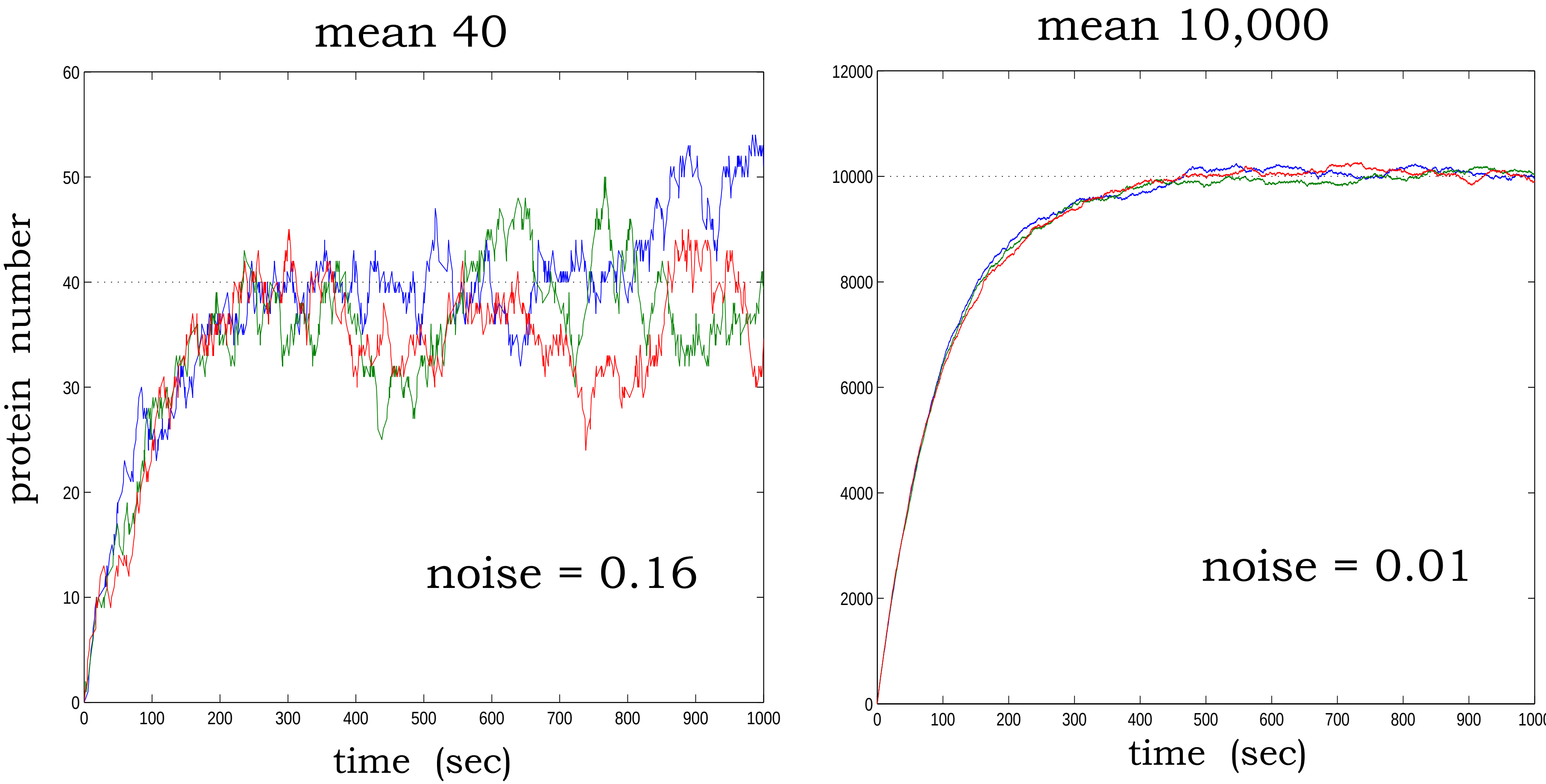
Both events are randomly affected by thermal fluctuations: collisions with other (solvent) molecules

When are chemical reactions significantly stochastic?

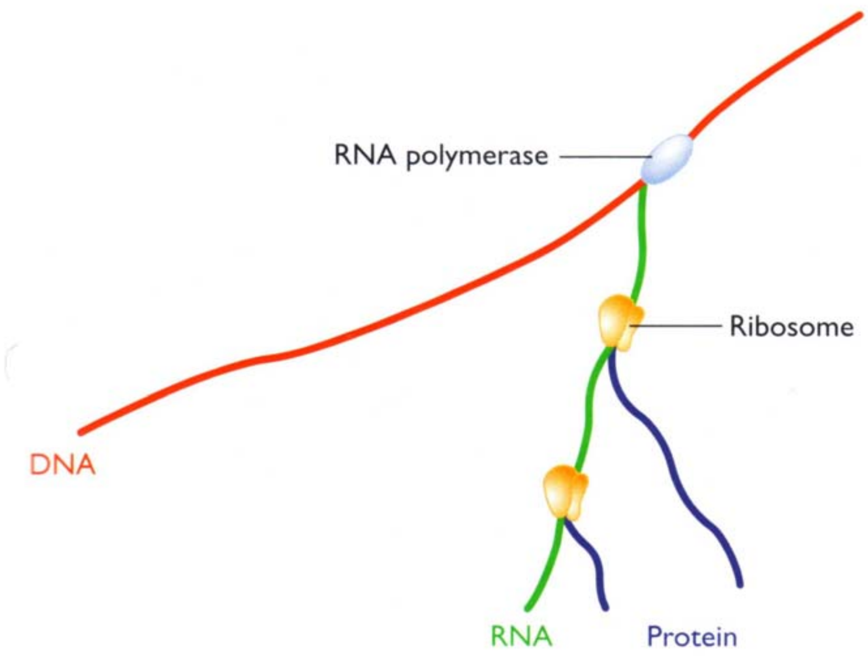
As a reaction only increases or decreases the number of molecules by one or two, it is only when numbers of molecules are small that random timing of individual reactions will matter.

$$\text{noise} = \frac{\text{standard deviation}}{\text{mean}}$$

Noise depends on low numbers.



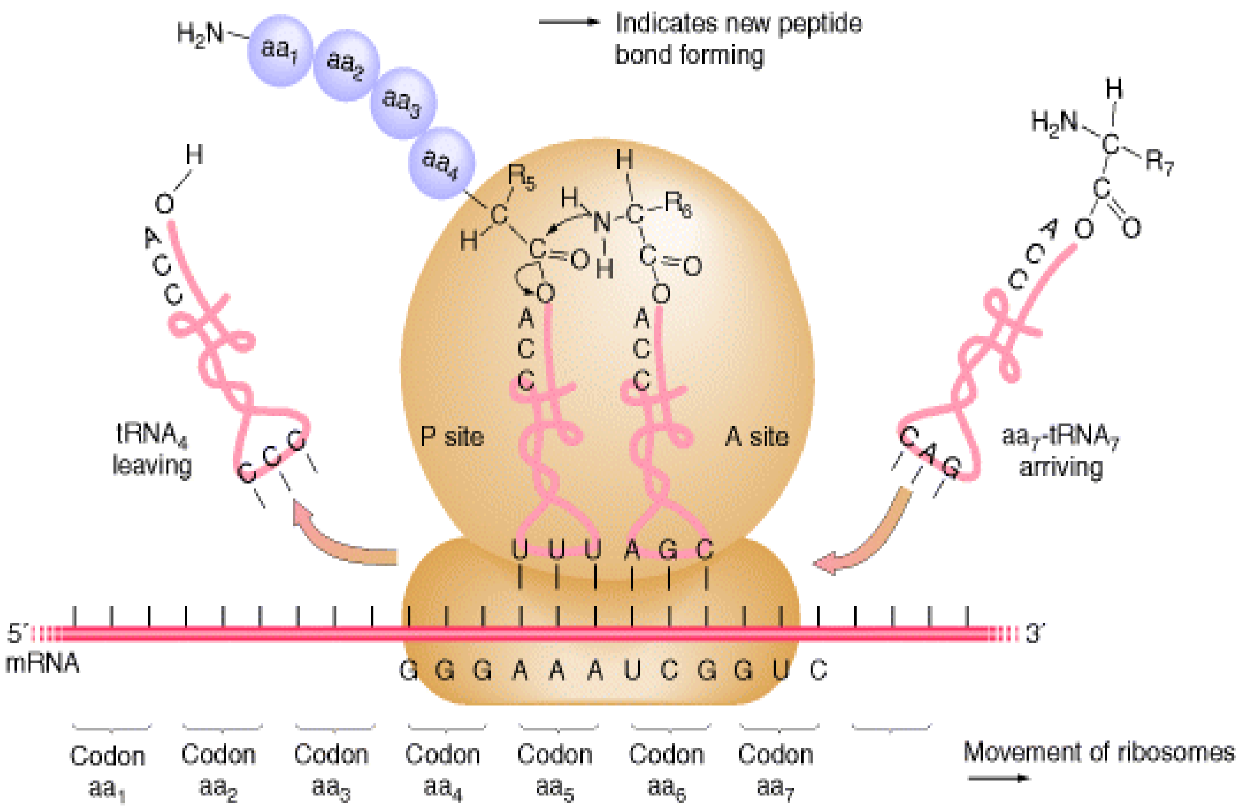
Gene expression in bacteria



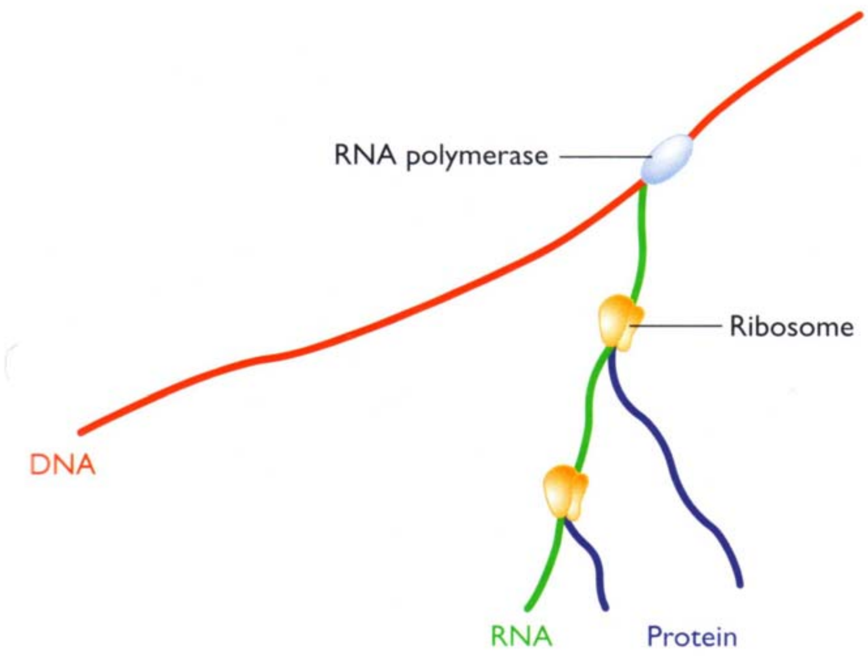
Transcription: DNA to mRNA

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

Translation: mRNA to protein



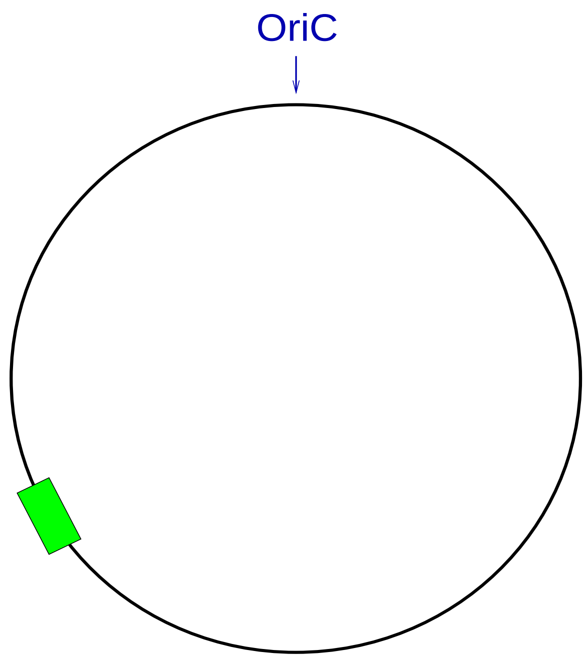
Gene expression in bacteria



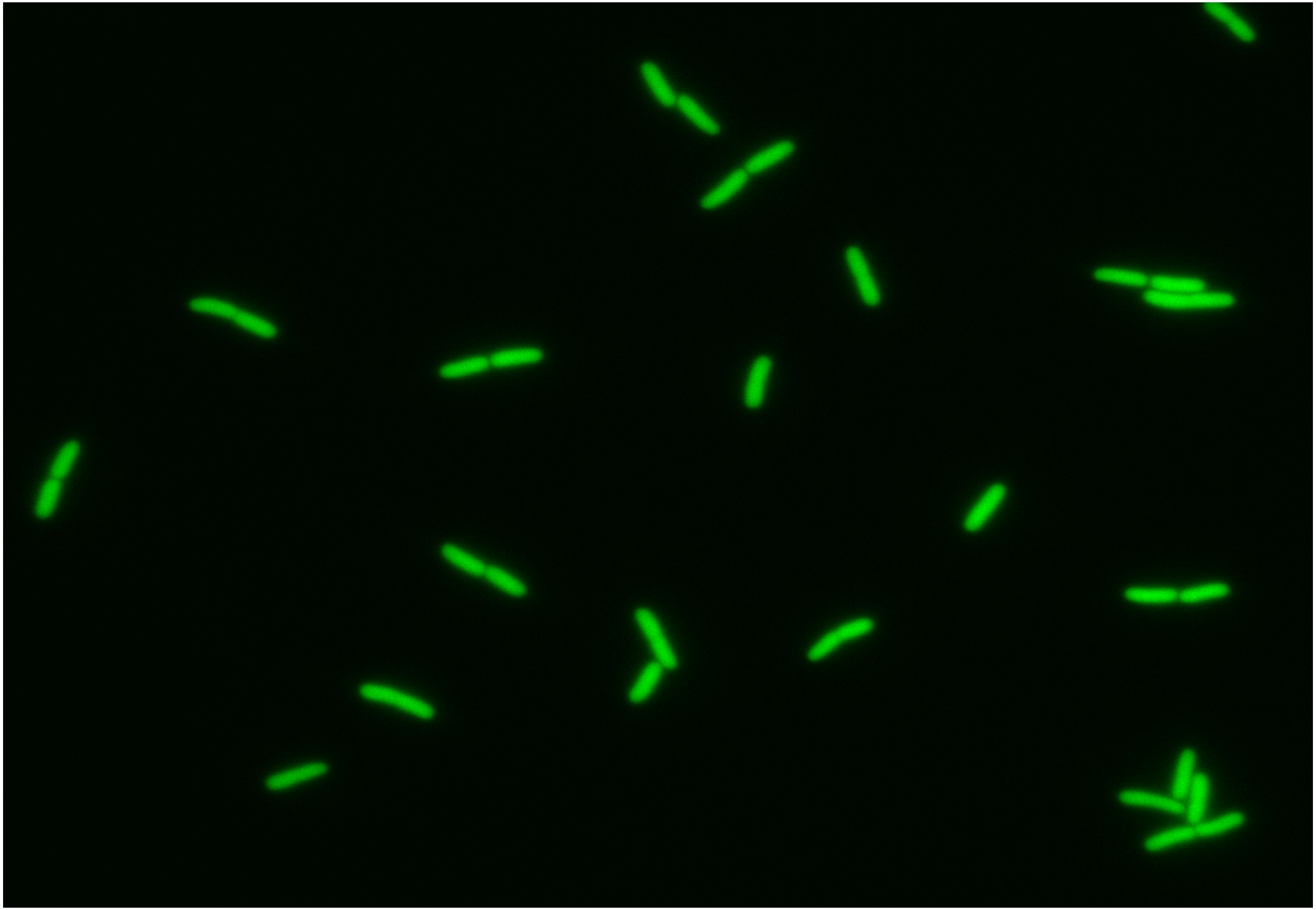
Measuring noise in single cells.

Measuring noise

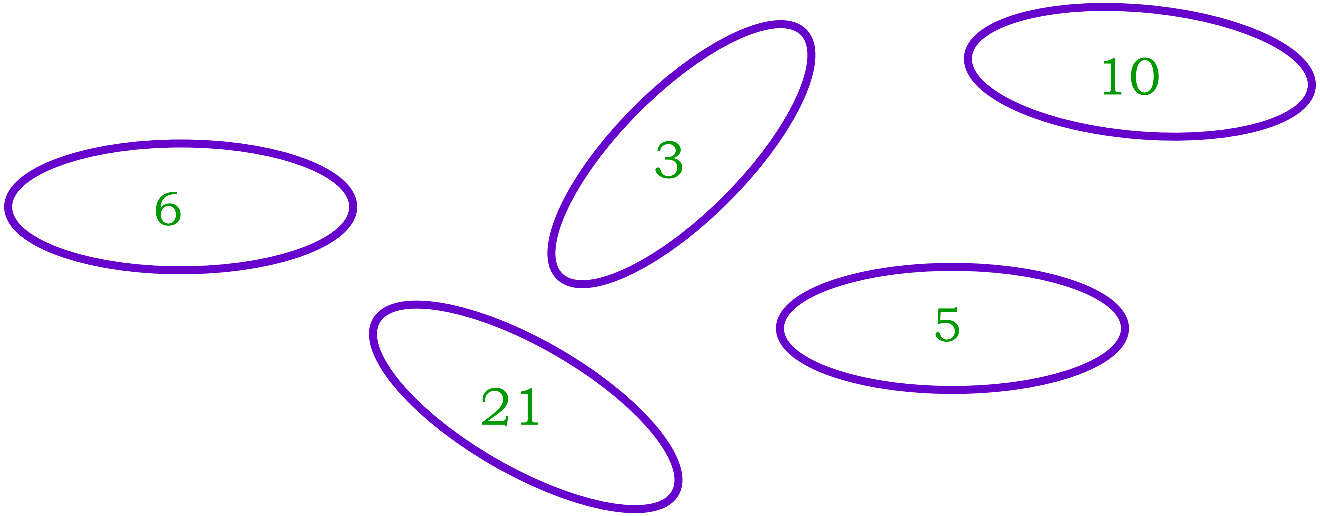
Integrate Green Fluorescent Protein



Escherichia coli
chromosome



Example: RNAP varies from cell to cell but gene expression is deterministic

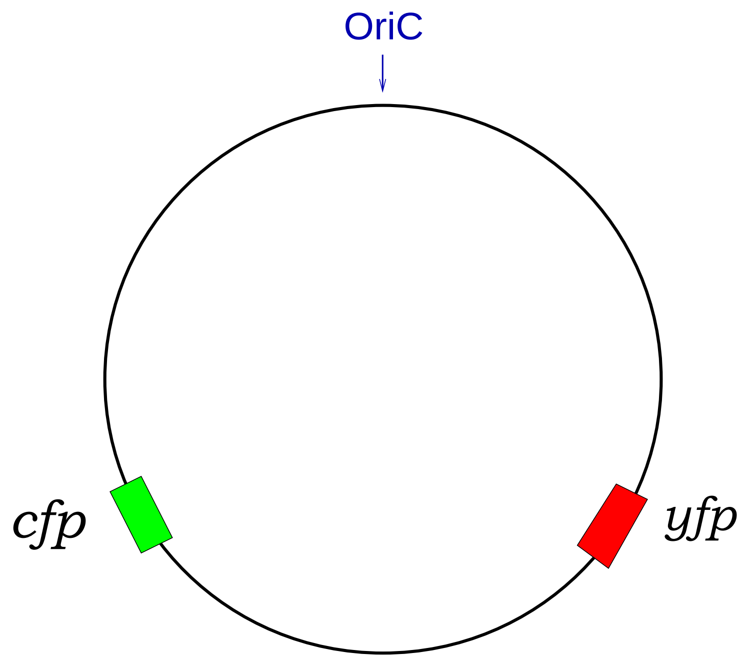


$$P \propto [\mathrm{RNAP}]$$

hence

$$\eta = \sqrt{\frac{\langle P^2 \rangle - \langle P \rangle^2}{\langle P \rangle^2}} = \sqrt{\frac{122.2 - 9^2}{9^2}} \approx 0.80$$

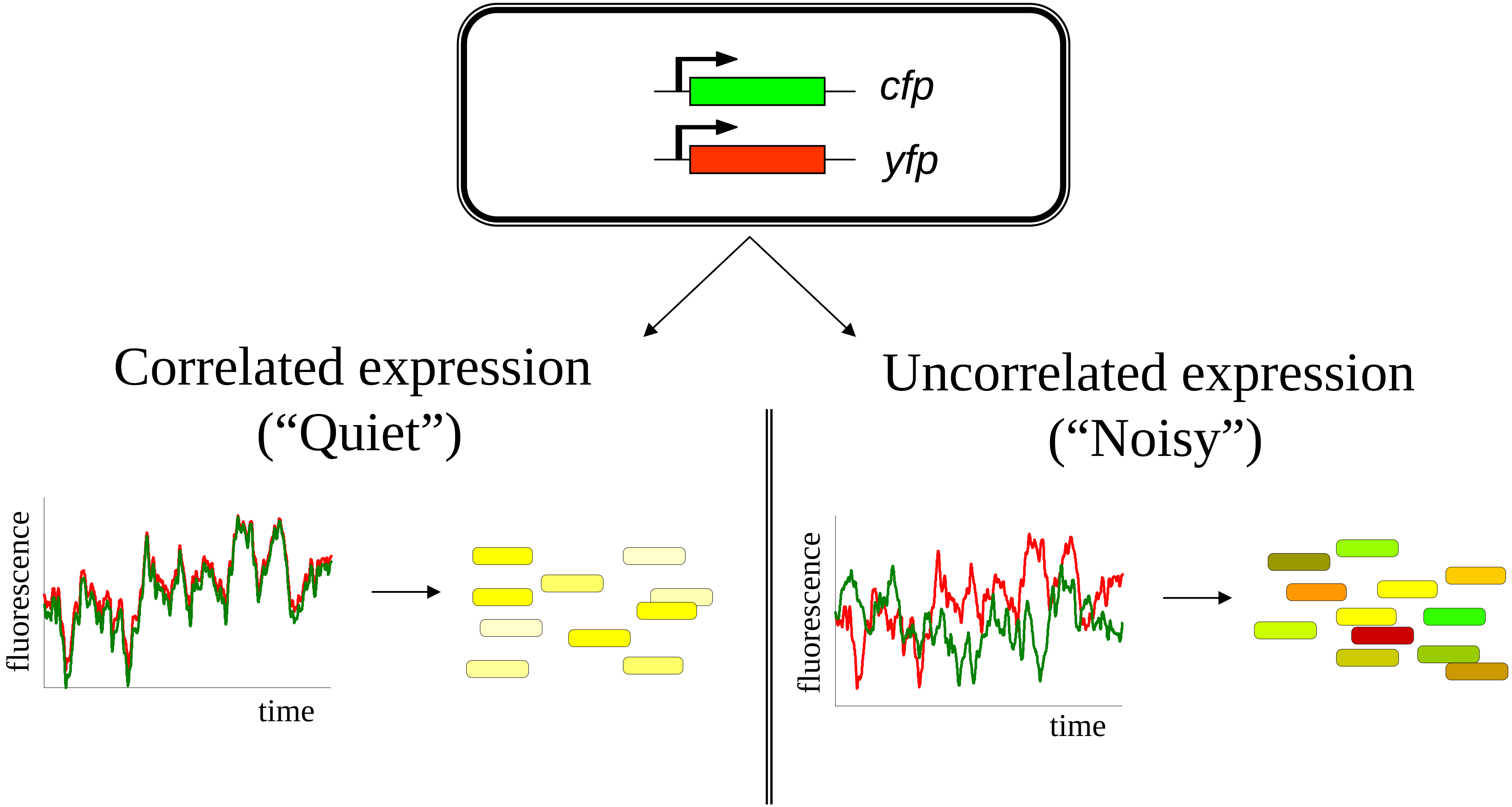
Two colour experiment



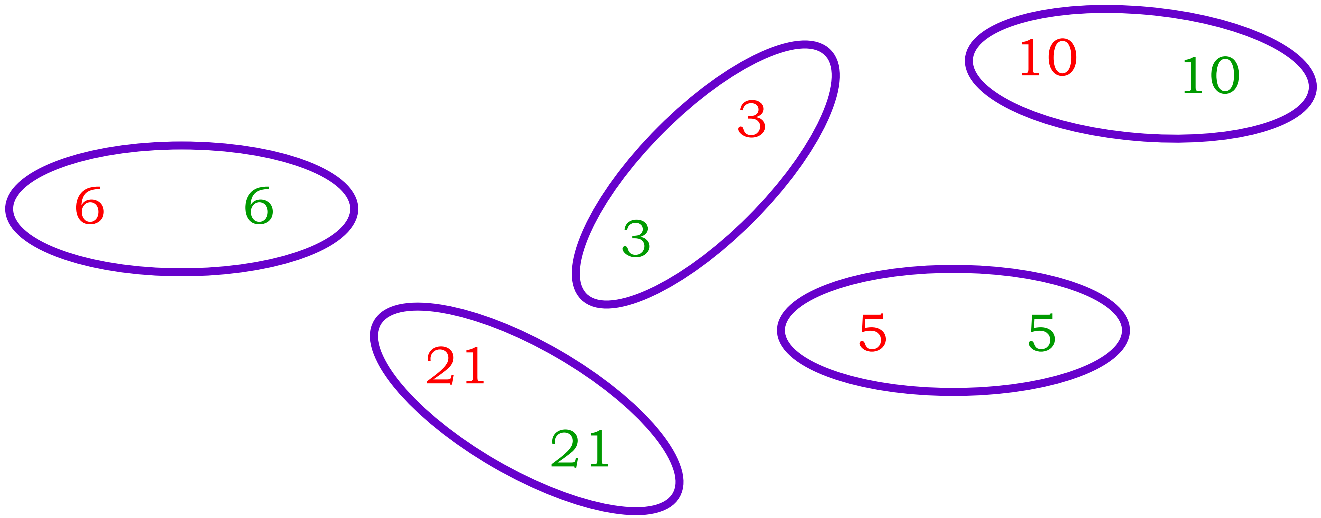
Look at *difference* between two colours

$$\eta_{\text{int}}^2 \propto (C - Y)^2$$

Each colour is controlled by identical regulatory sequences.



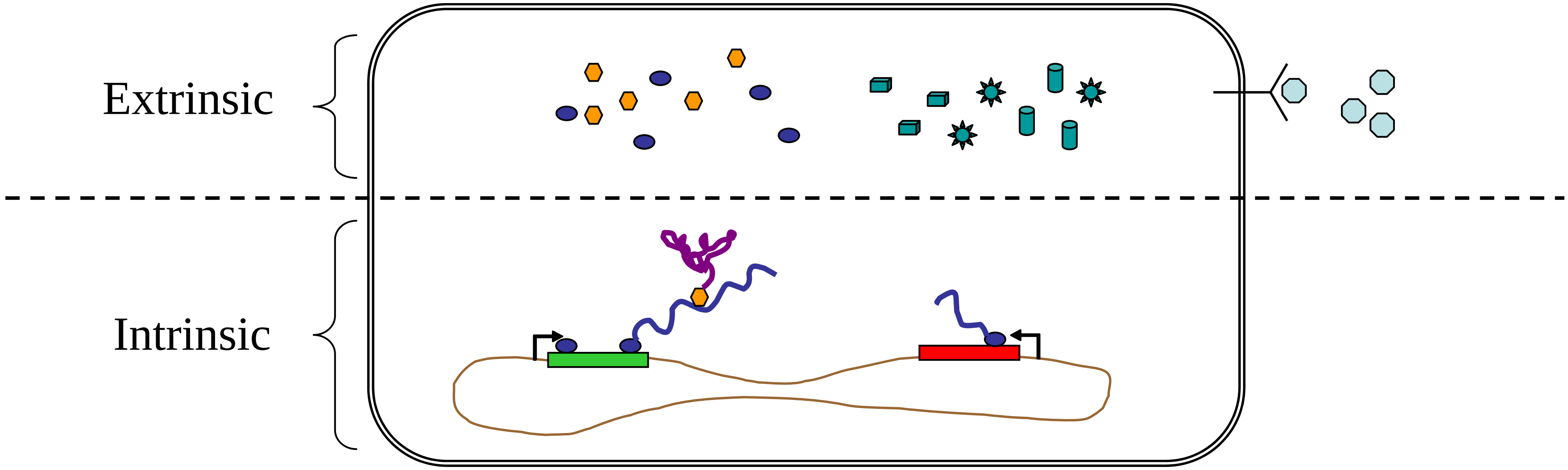
Example: RNAP varies but gene expression is deterministic



No intrinsic noise

$$\eta_{\text{int}}^2 = \frac{\frac{1}{2N}\sum (C-Y)^2}{\left(\frac{1}{N}\sum C\right)\left(\frac{1}{N}\sum Y\right)} = 0$$

Two types of noise: intrinsic and extrinsic

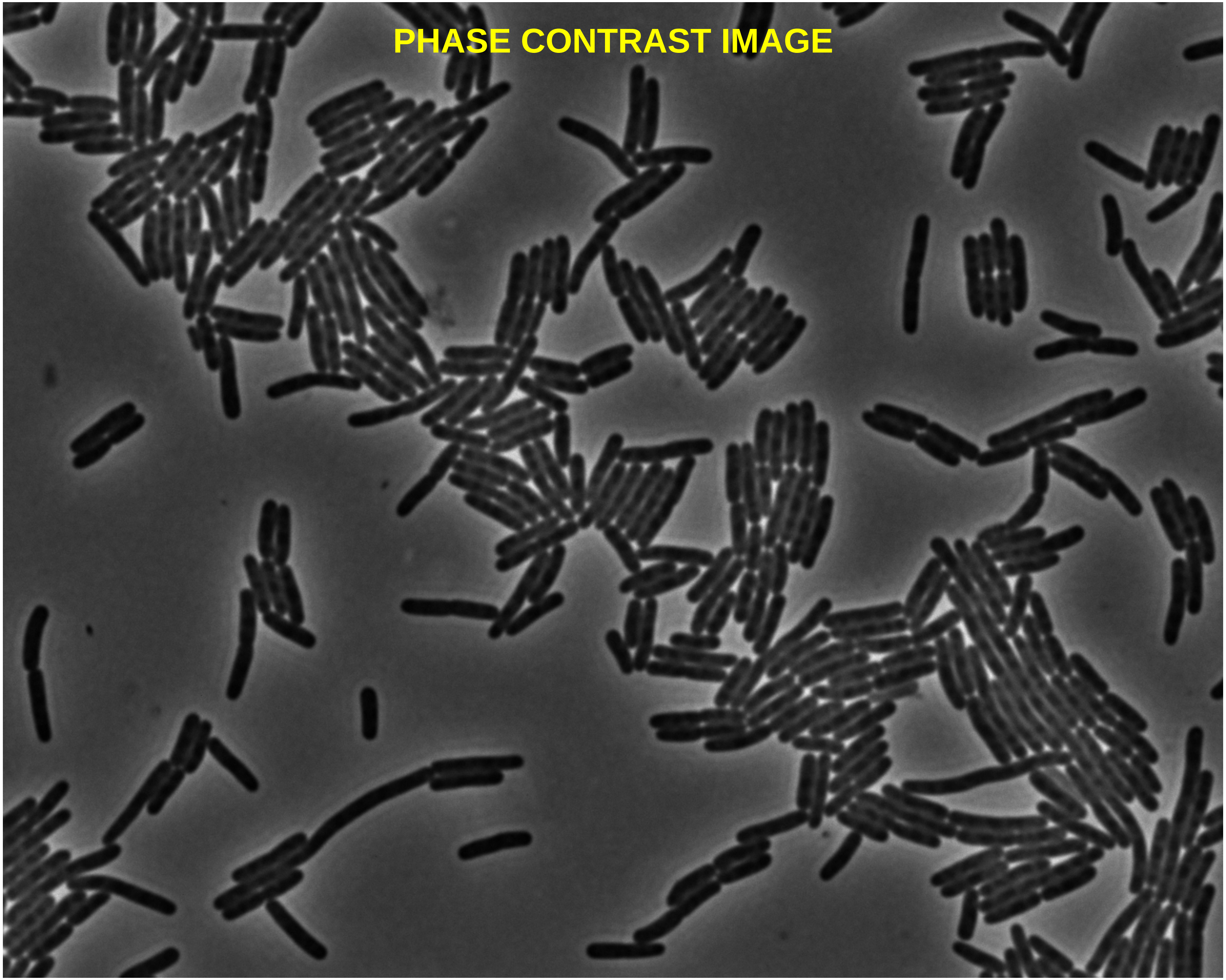


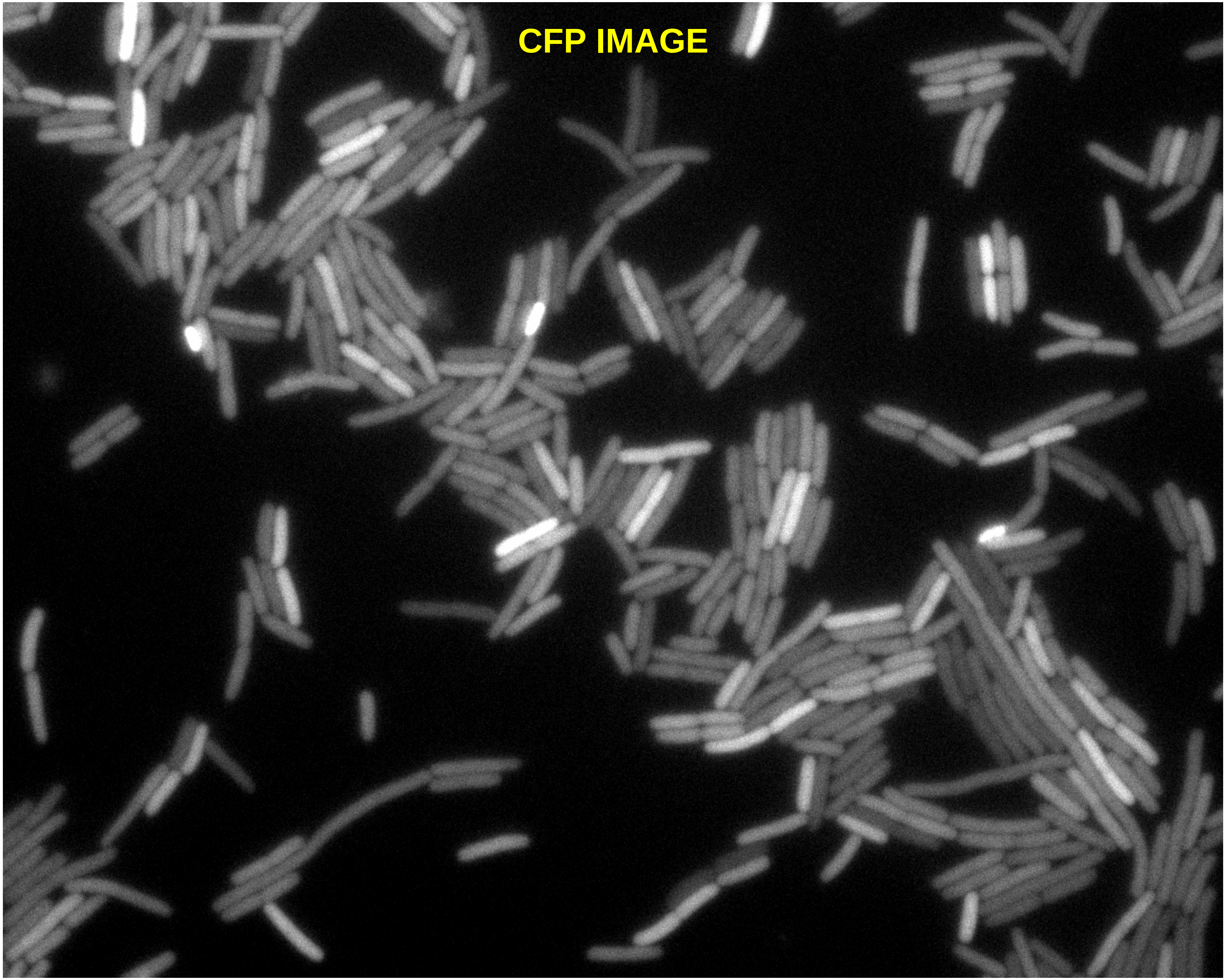
Extrinsic variables: same for each gene

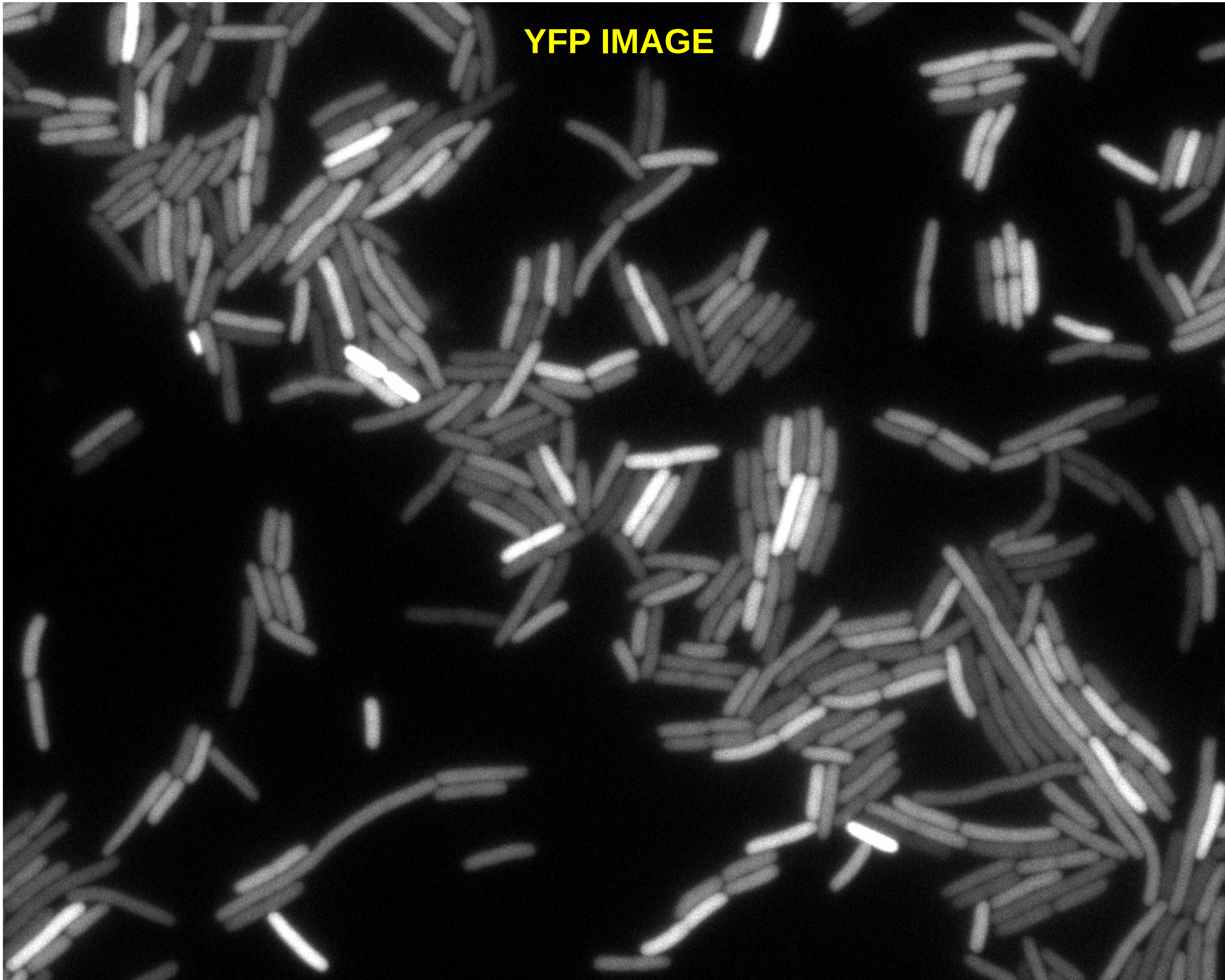
e.g. number of free RNAPs, cellular environment

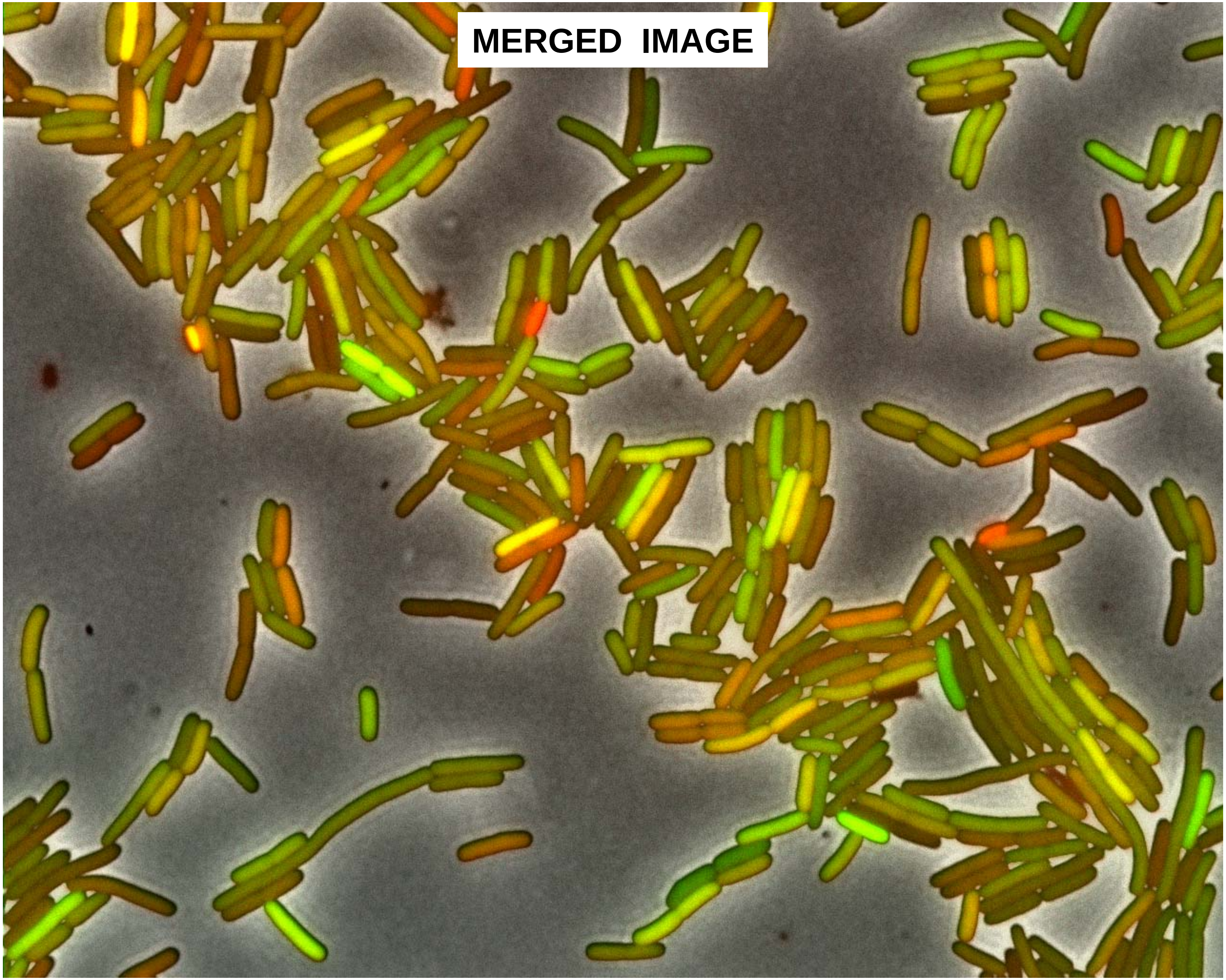
Intrinsic variables: different for each gene

e.g. number of transcribing RNAPs, number of mRNAs


PHASE CONTRAST IMAGE

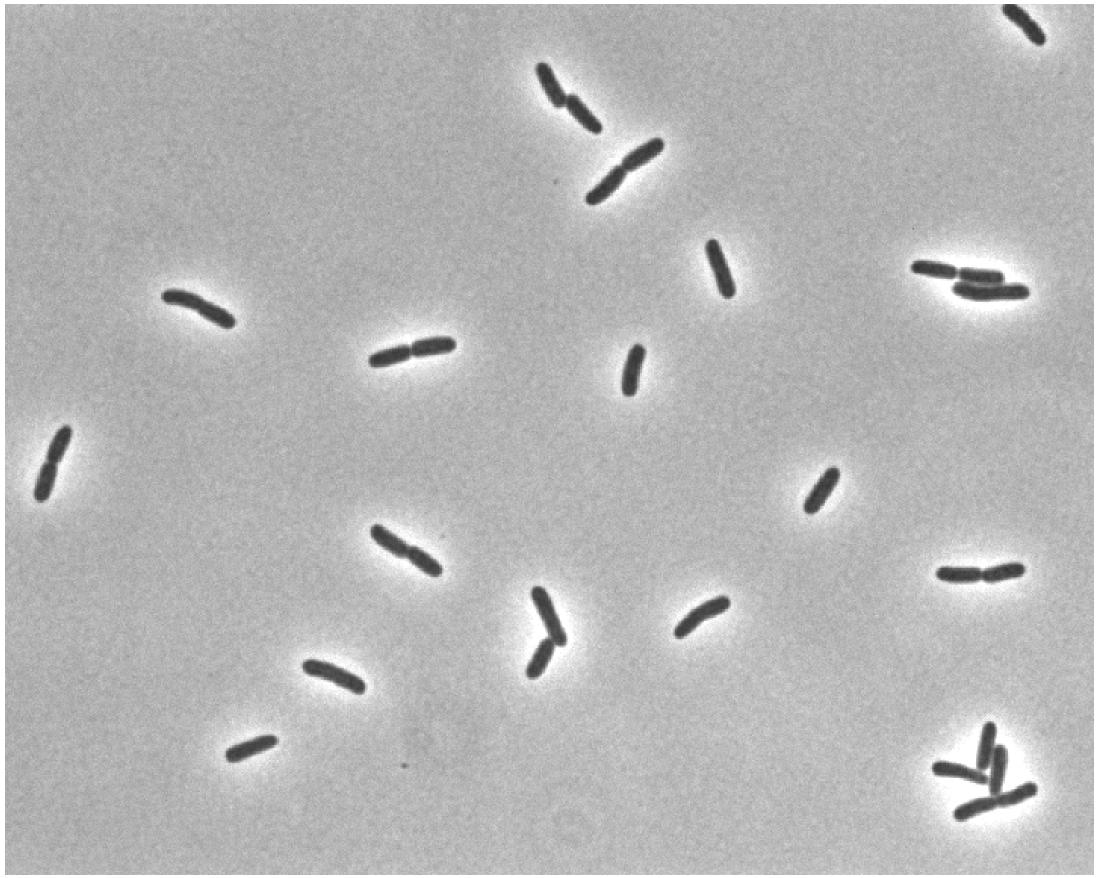

CFP IMAGE


YFP IMAGE

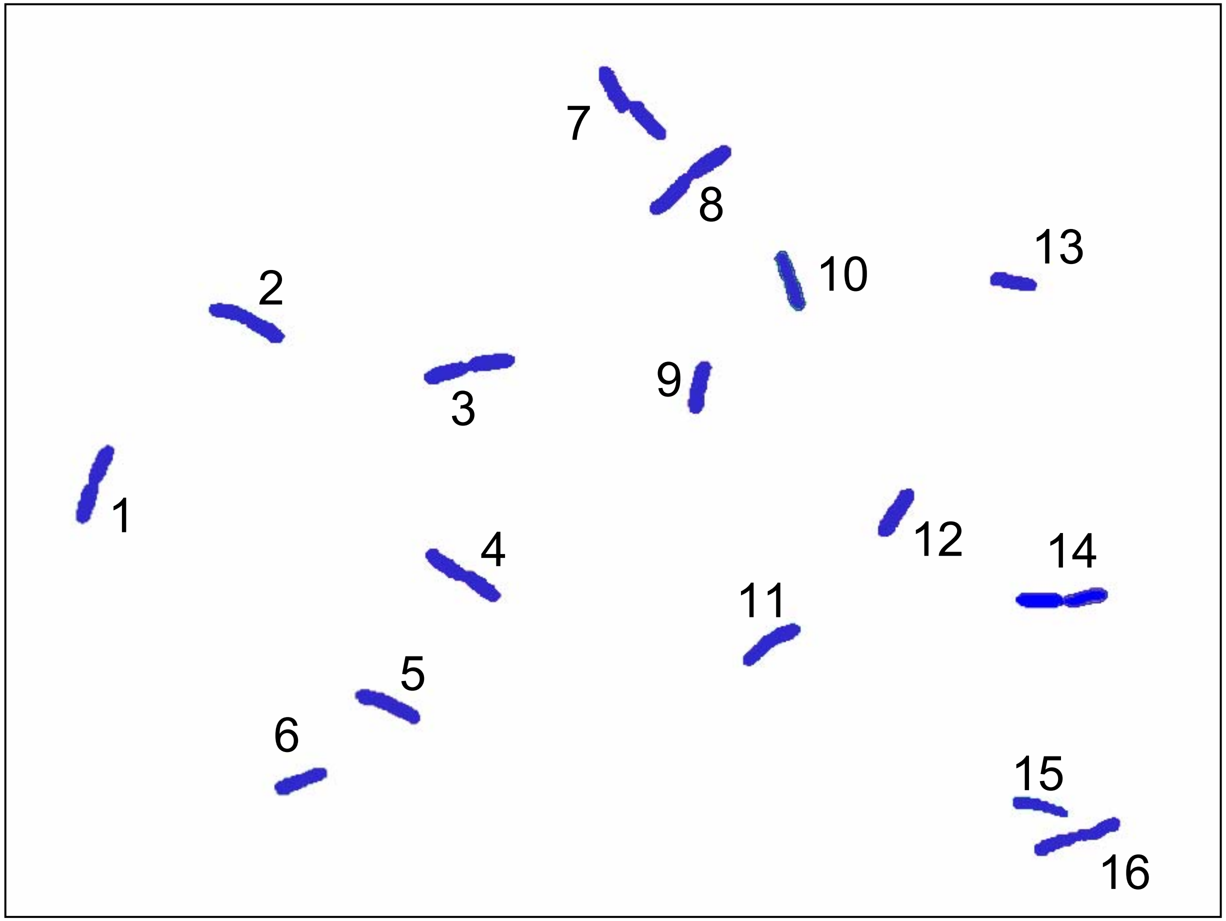

MERGED IMAGE

Extracting fluorescence values

Phase image

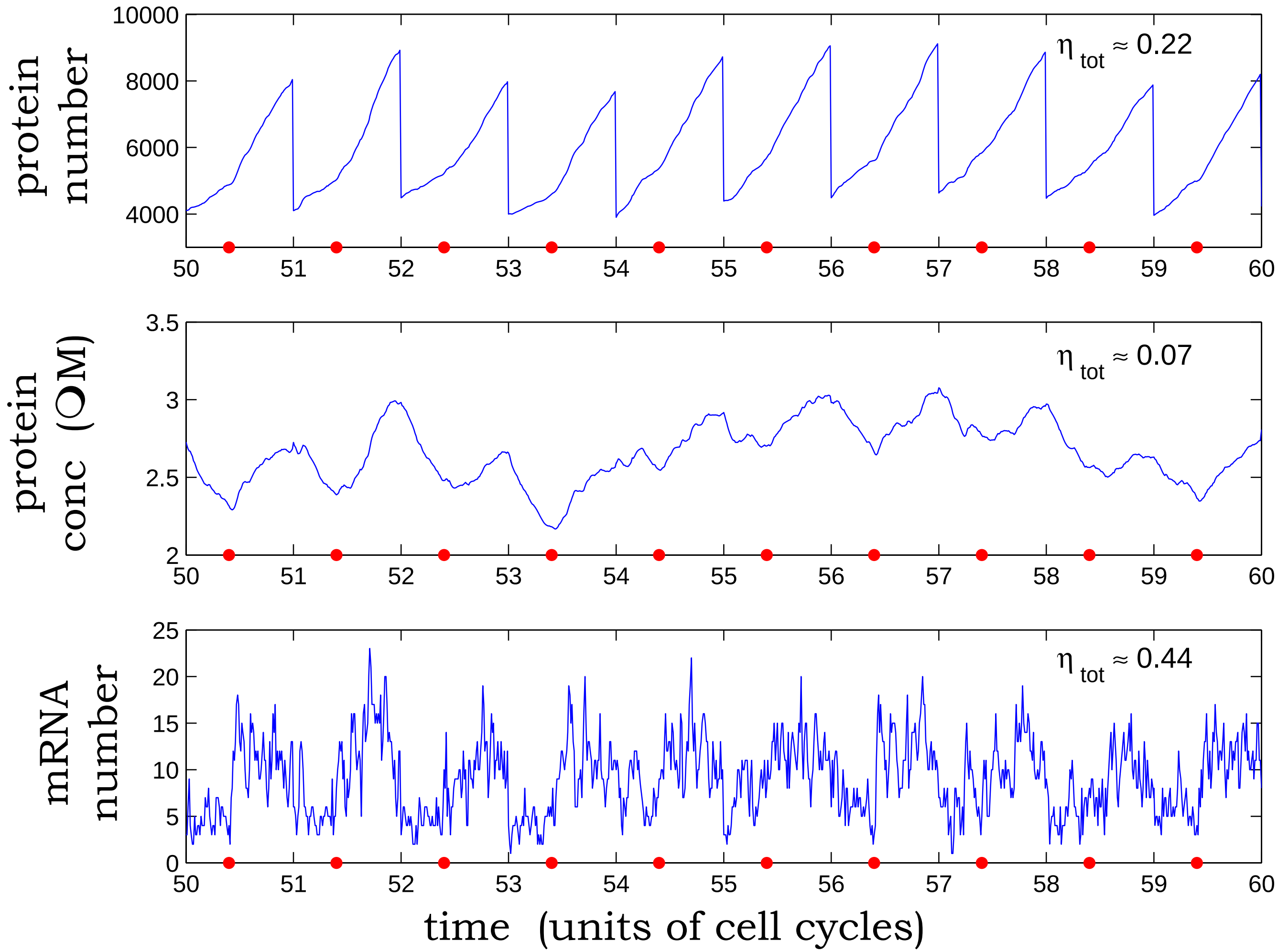


Automatic cell identification

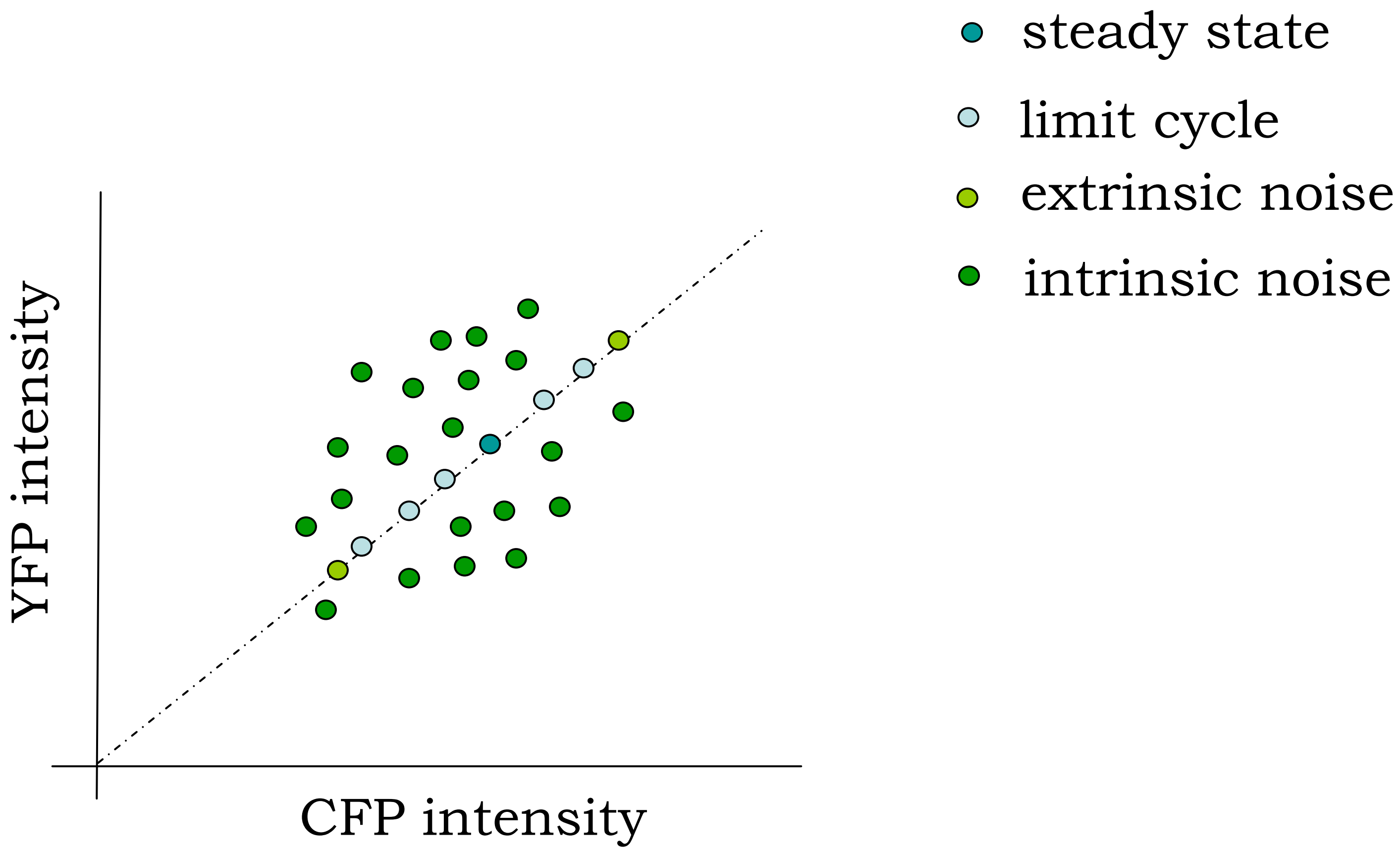


What results do we expect?

Simulation of constitutive gene expression
(Gillespie method)



Experimental results



Experimentally

$$\frac{1}{N}\sum_{k=1}^{N} P_k^m = \overline{\langle P^m \rangle}$$

extrinsic average (overline)

intrinsic average (angle brackets)

then

$$\eta_{\text{tot}}^2 = \frac{(\text{standard deviation})^2}{(\text{mean})^2}$$

$$= \frac{\overline{\langle P^2 \rangle} - \left(\overline{\langle P \rangle}\right)^2}{\left(\overline{\langle P \rangle}\right)^2}$$

$$= \frac{\overline{\langle P^2 \rangle} - \overline{\langle P \rangle^2} + \overline{\langle P \rangle^2} - \left(\overline{\langle P \rangle}\right)^2}{\left(\overline{\langle P \rangle}\right)^2}$$

$$= \frac{\overline{\langle P^2 \rangle - \langle P \rangle^2}}{\left(\overline{\langle P \rangle}\right)^2} + \frac{\overline{\langle P \rangle^2} - \left(\overline{\langle P \rangle}\right)^2}{\left(\overline{\langle P \rangle}\right)^2}$$

giving

$$\eta_{\text{tot}}^2 = \eta_{\text{int}}^2 + \eta_{\text{ext}}^2$$

Definitions

total noise

bracket denotes average over all cells

$$\eta_{\mathrm{tot}}^2 = \frac{\langle C^2 + Y^2 \rangle - 2\langle C \rangle \langle Y \rangle}{2\langle C \rangle \langle Y \rangle}$$

intrinsic and extrinsic noise

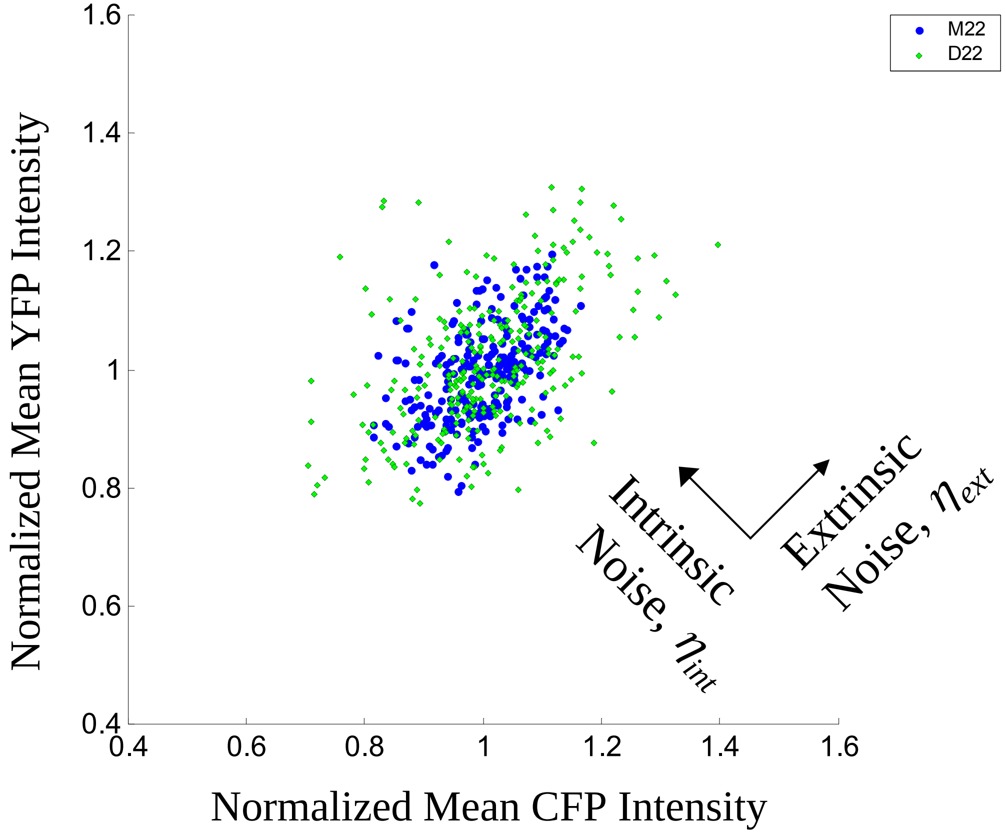
$$\eta_{\mathrm{int}}^2 = \frac{\langle (C - Y)^2 \rangle}{2\langle C \rangle \langle Y \rangle}$$

$$\eta_{\mathrm{ext}}^2 = \frac{\langle CY \rangle - \langle C \rangle \langle Y \rangle}{\langle C \rangle \langle Y \rangle}$$

which obey

$$\boxed{\eta_{\mathrm{tot}}^2 = \eta_{\mathrm{int}}^2 + \eta_{\mathrm{ext}}^2}$$

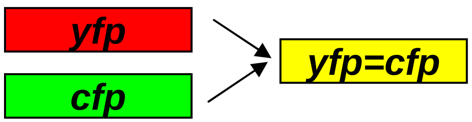
Experimentally, two different noise sources can be distinguished.



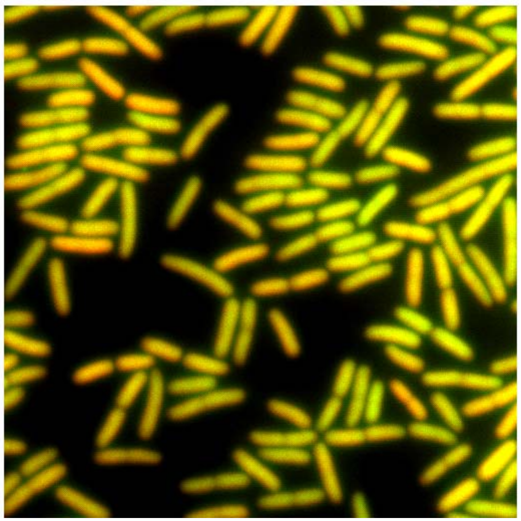
Constraint: $\eta_{int}^2 + \eta_{ext}^2 = \eta_{tot}^2$

Intrinsic noise can be small

Twin (strong) lac-regulated artificial promoters, based on λ P_L

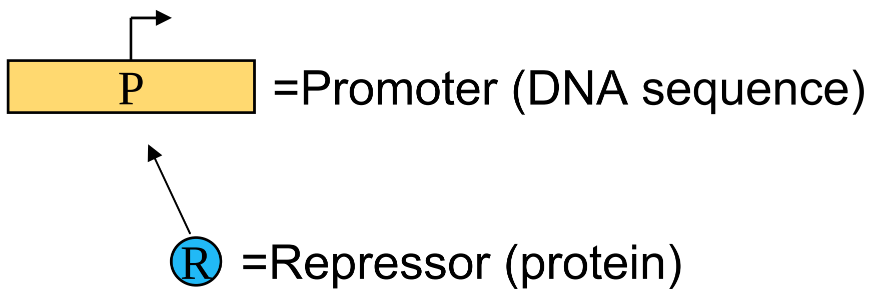


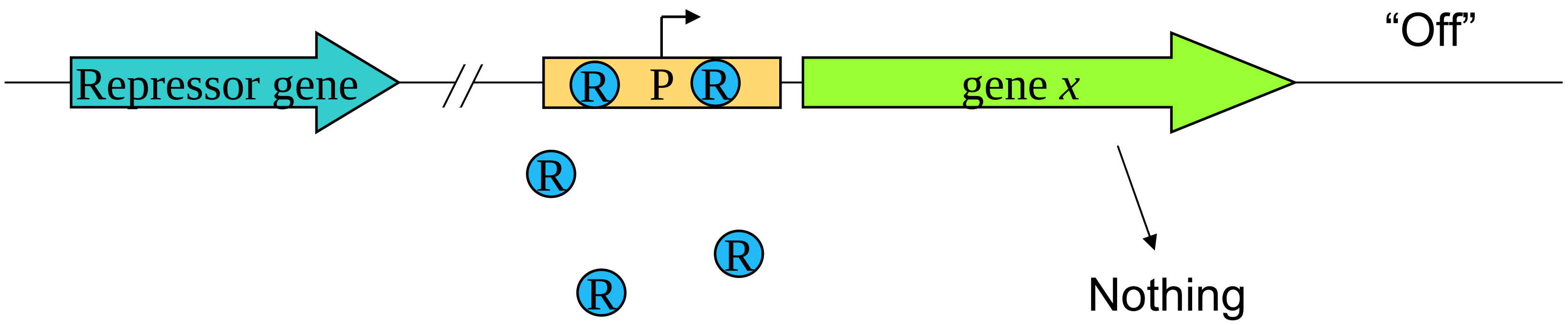
ΔlacI



Intensity	≡ 1.0
η_{int}	= 0.055 (0.051-0.06)
η_{ext}	= 0.054 (0.048-0.059)
η_{tot}	= 0.077 (0.074-0.081)

Negative transcriptional control



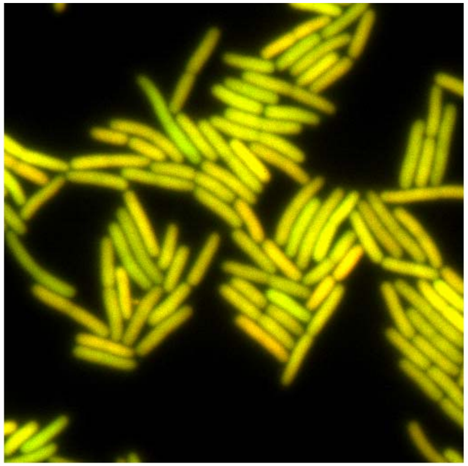


Intrinsic noise depends on expression level

yfp

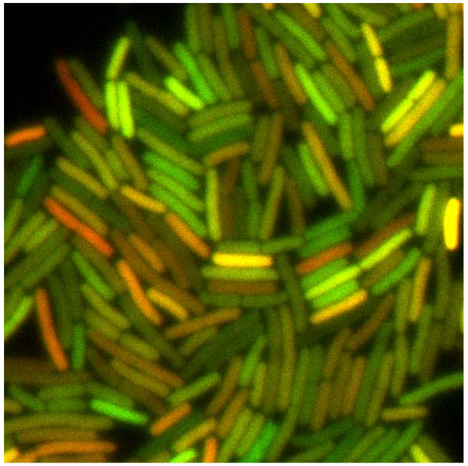
cfp

WT $_{(RPR37)}$ + IPTG



decrease expression 32-fold

WT $_{(RPR37)}$



Intensity = 1
η_{int} = 0.063 (0.058-0.069)
η_{ext} = 0.098 (0.09-1.1)

Intensity = 0.03
η_{int} = 0.25 (0.22-0.27)
η_{ext} = 0.32 (0.3-0.35)

1. Gene expression is both intrinsically and extrinsically stochastic.

2. Extrinsic noise is usually greater than intrinsic noise.

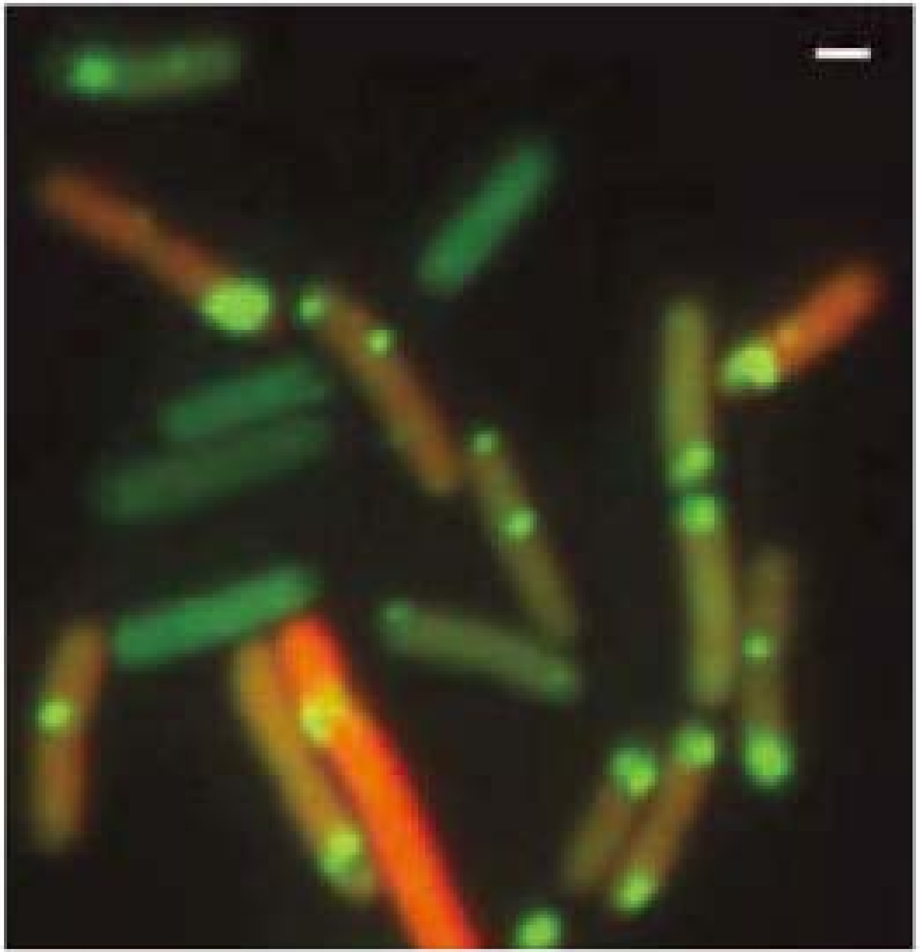
3. Intrinsic noise decreases as gene expression levels increase.

Elowitz *et al.* (2002)
Swain *et al.* (2002)

Real-Time Kinetics of Gene Activity in Individual Bacteria

Ido Golding,[1,*] Johan Paulsson,[2,3] Scott M. Zawilski,[1] and Edward C. Cox[1,*]

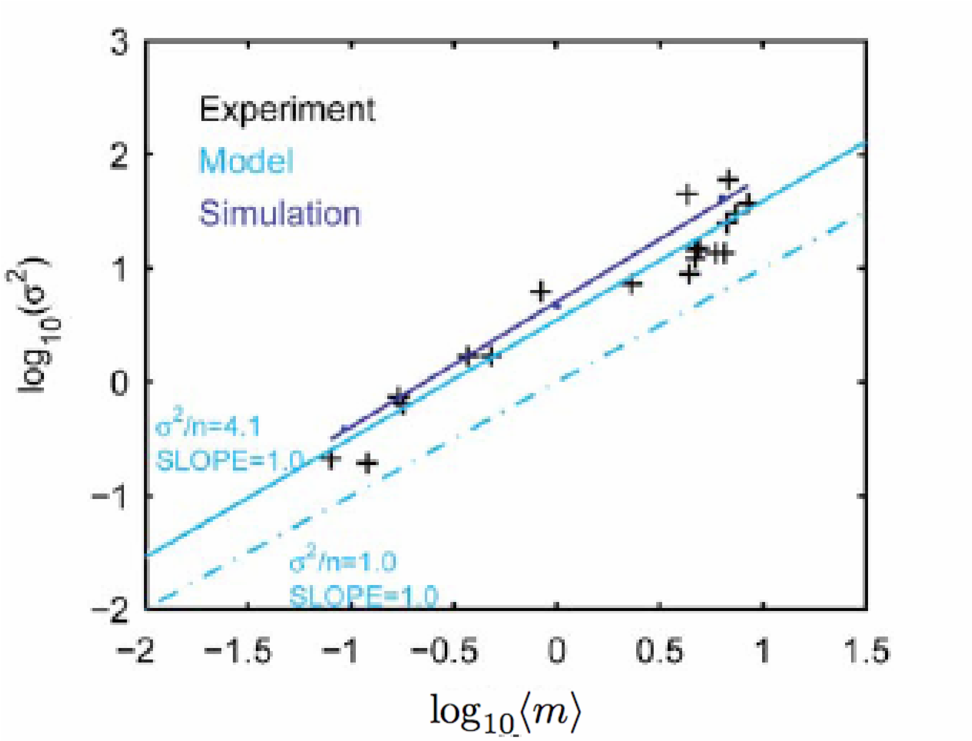
Cell *123*, 1025–1036, December 16, 2005



Red: protein
Green spots: mRNA

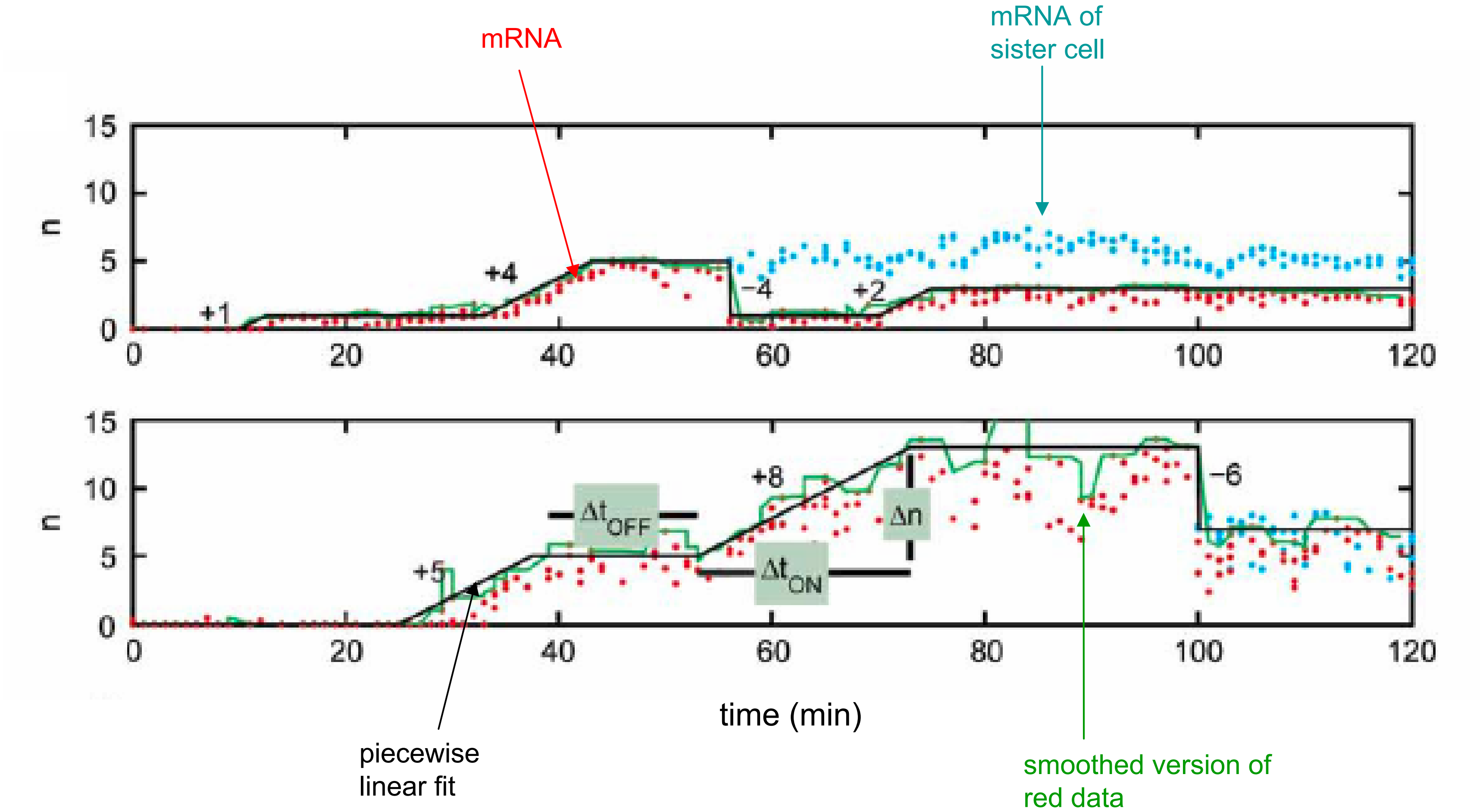
scale bar: 1 μm

Variance in mRNA against mRNA numbers

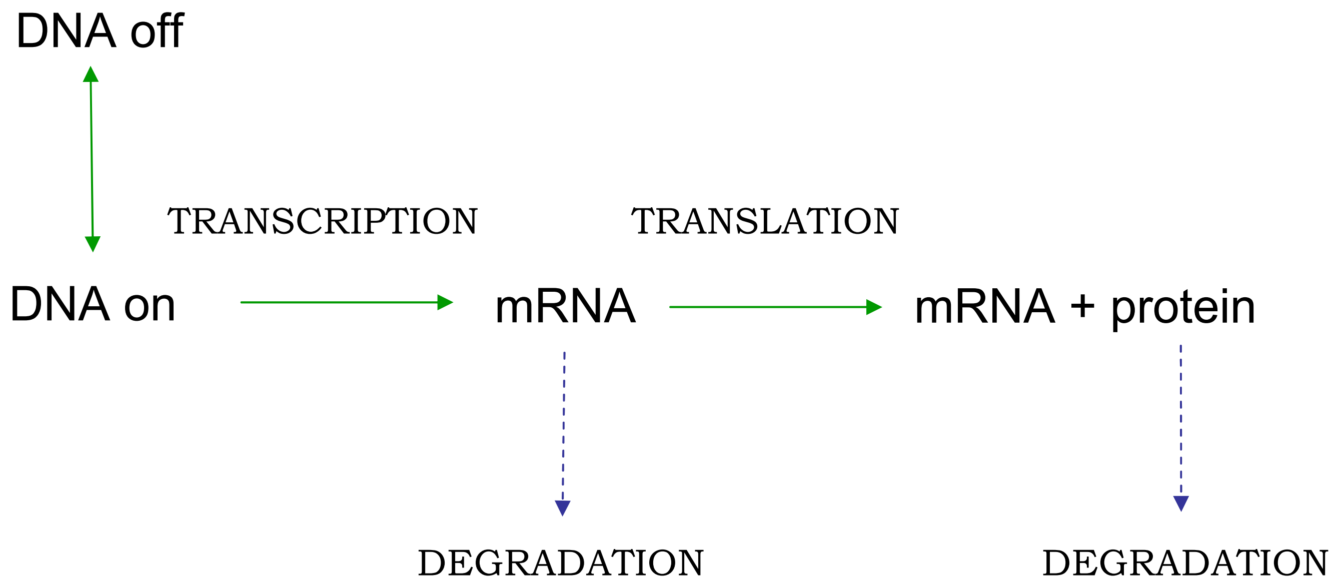


Naïve prediction: $\sigma_m^2 = \langle m \rangle$

Time course of mRNA numbers: mRNA is produced in bursts



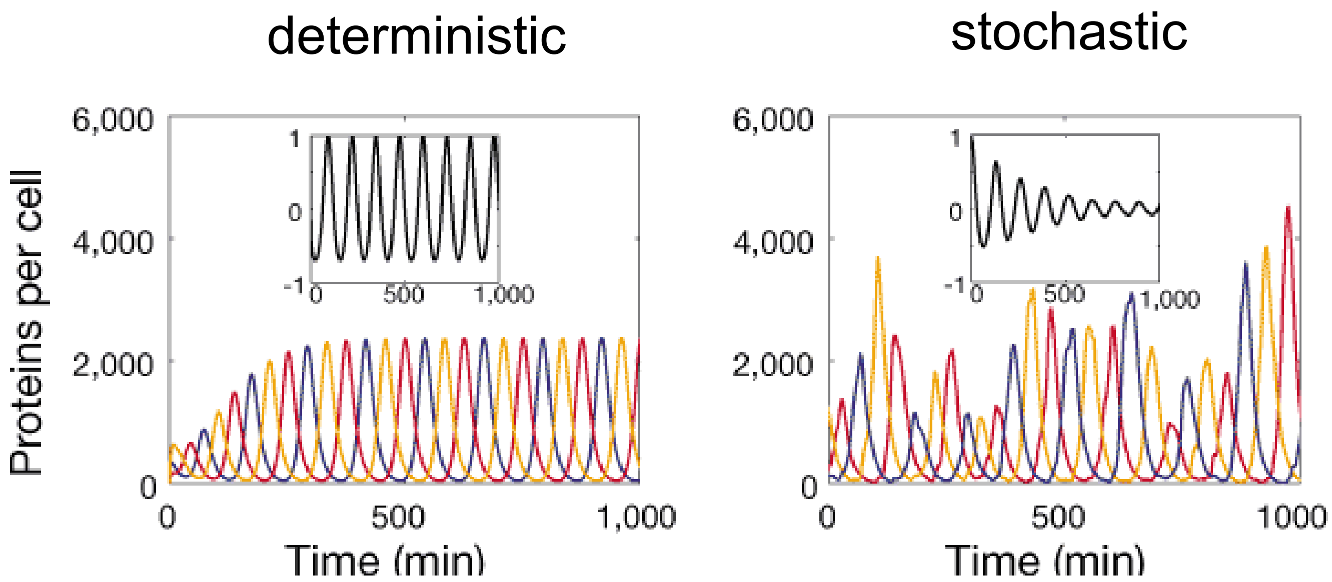
More complex model



Consequences…

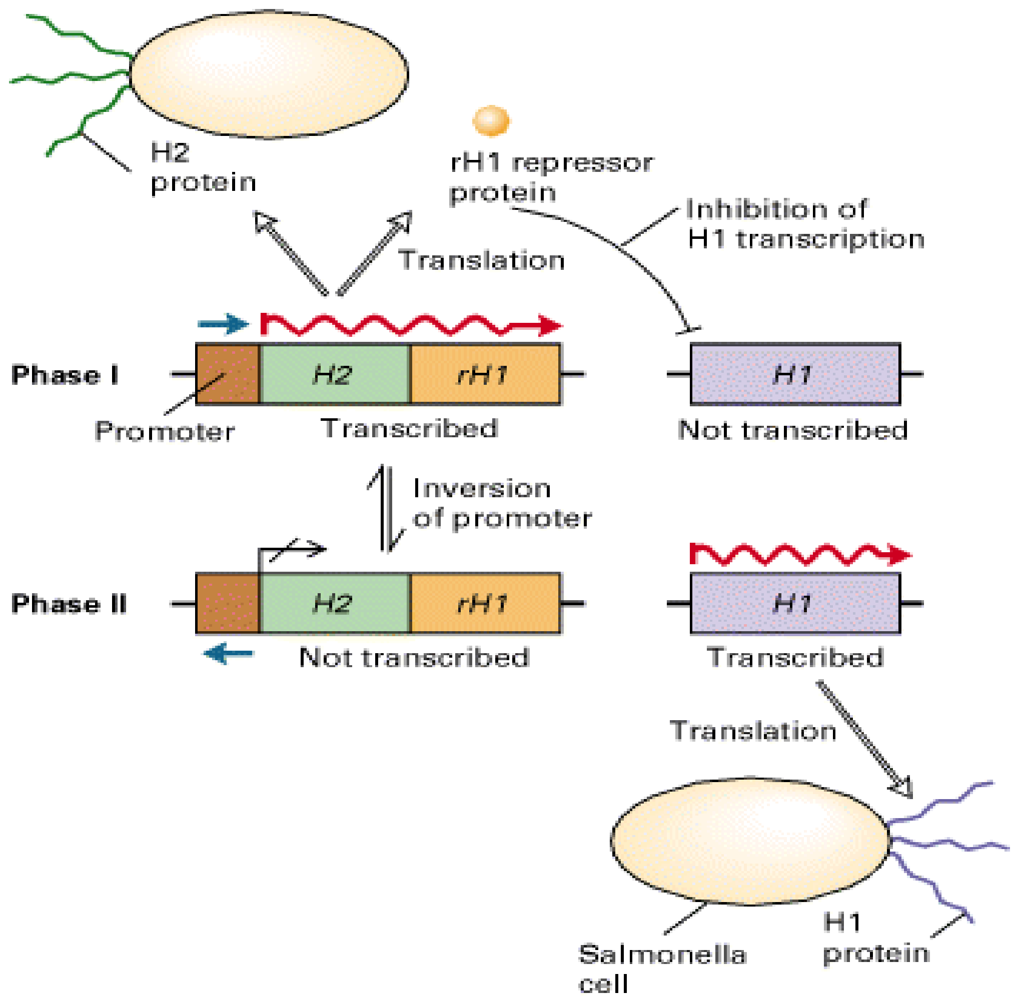
Noise affects the reliable function of genetic networks, by affecting *timing.*

e.g. biological rhythms
(Elowitz & Leibler, Barkai & Leibler)



Noise can also be exploited by cells, e.g. avoiding the immune response.

Salmonella typhimurium



from Molecular Cell Biology, 4th ed