Life at the Single-Cell Level

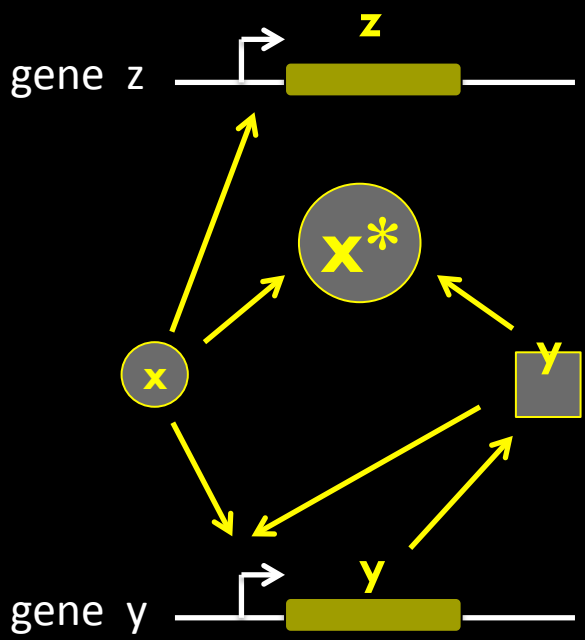
Michael Elowitz
HHMI/Caltech

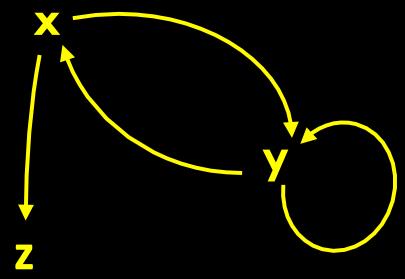
Bacterial cells

Consider a small rod-shaped bacterium

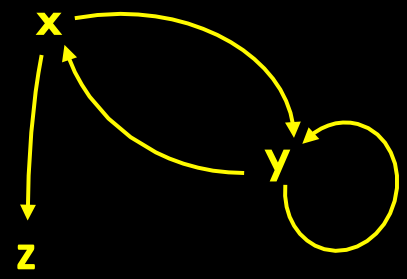
Consider a small rod-shaped bacterium

Gene circuits involve specific interactions between genes and proteins



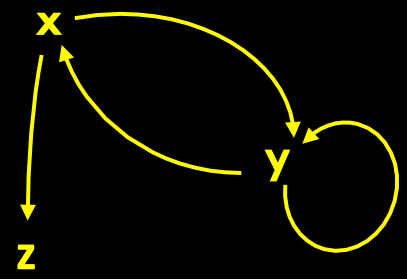


Gene circuits involve specific interactions between genes and proteins



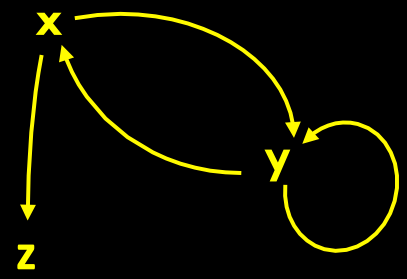
Difficulties in Understanding Gene Circuits

- Circuits are dynamic



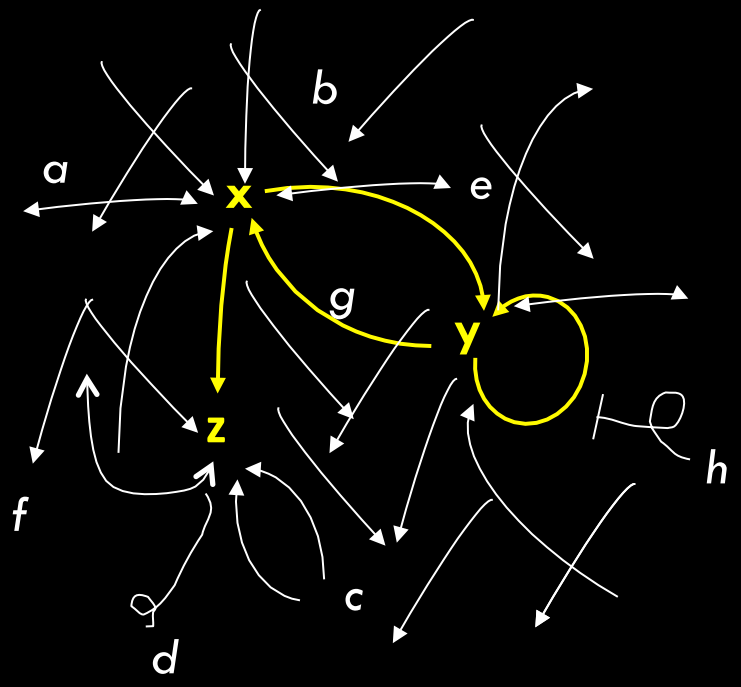
Difficulties in Understanding Gene Circuits

- Circuits are dynamic
- Circuits are “noisy”



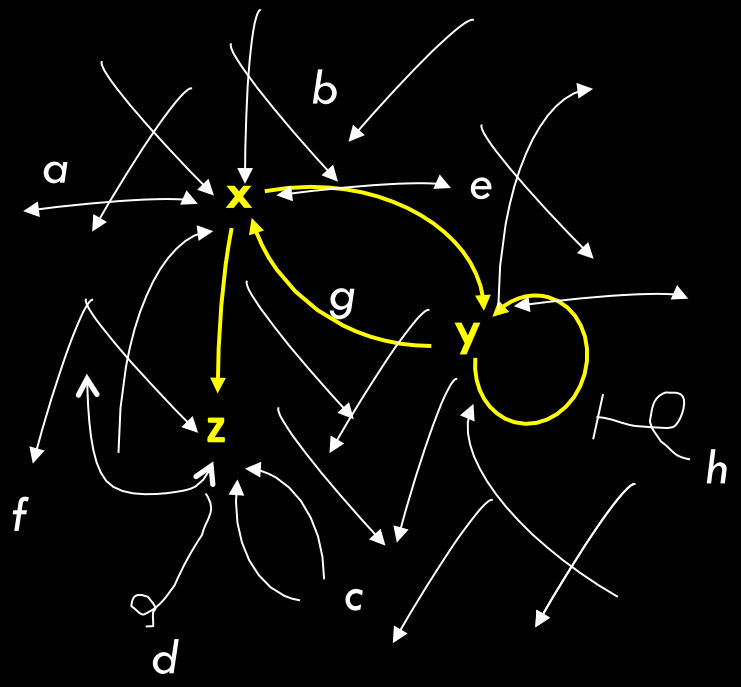
Difficulties in Understanding Gene Circuits

- Circuits are dynamic
- Circuits are “noisy”
- Circuit are complex

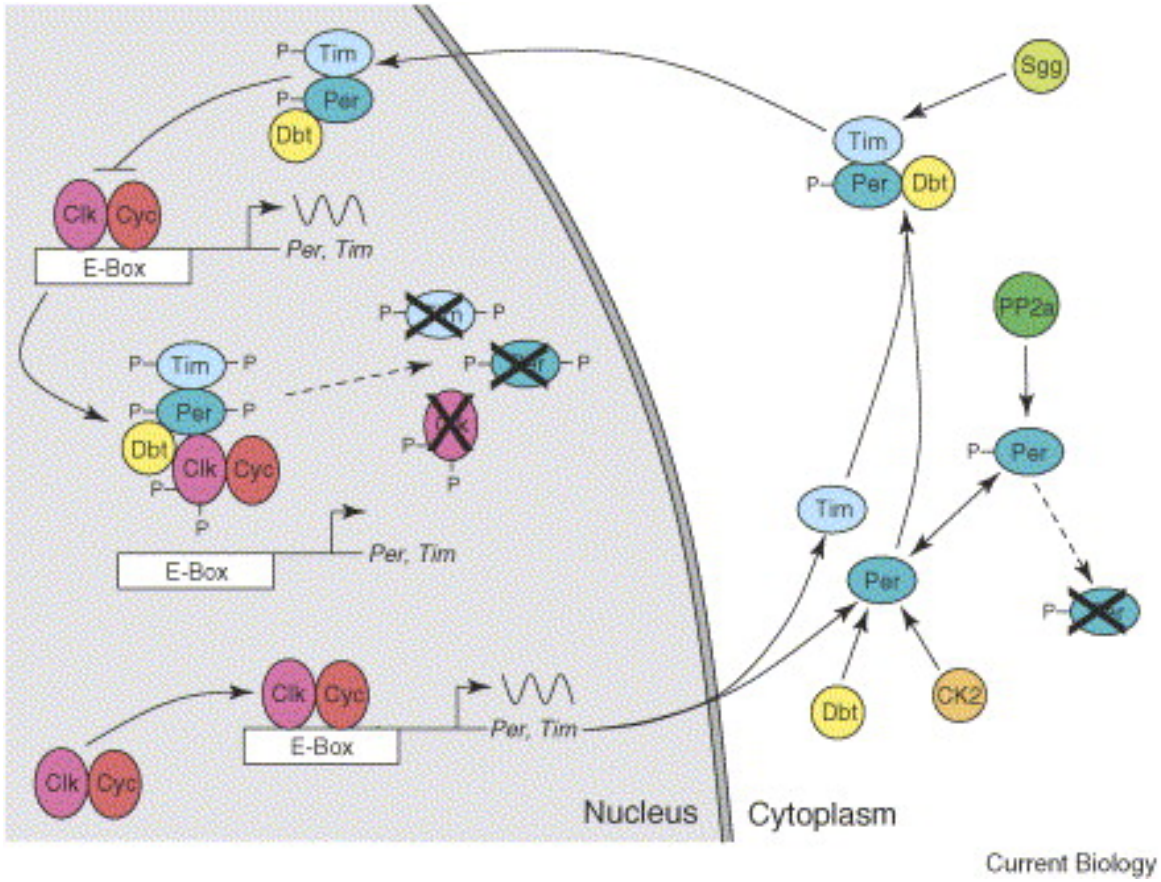


Approaches to Understanding Gene Circuits

- Use Synthetic Biology to Construct Simple Gene Circuits that Program Cellular Behavior
- Use Movies to Analyze Gene Circuits at the Single-Cell Level.
- Goal: Quantitative understanding of gene circuit design principles

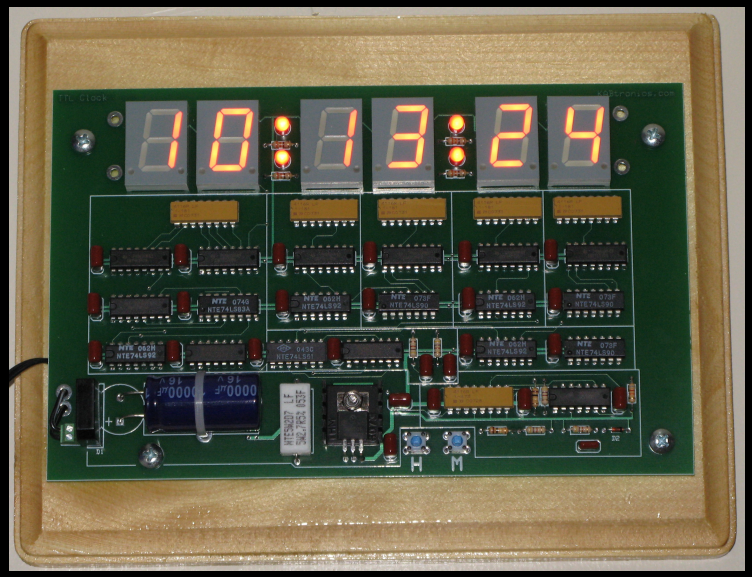


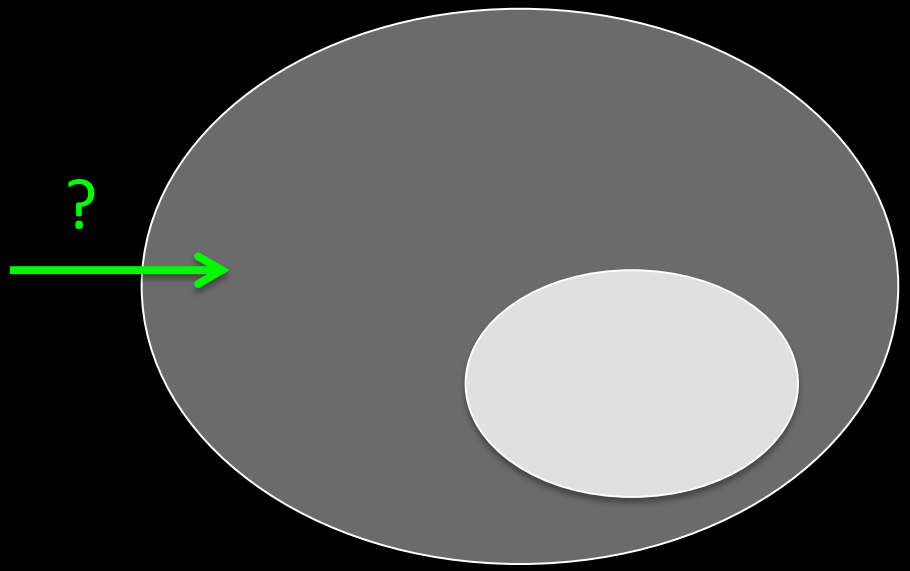
Cells have evolved accurate clock circuits



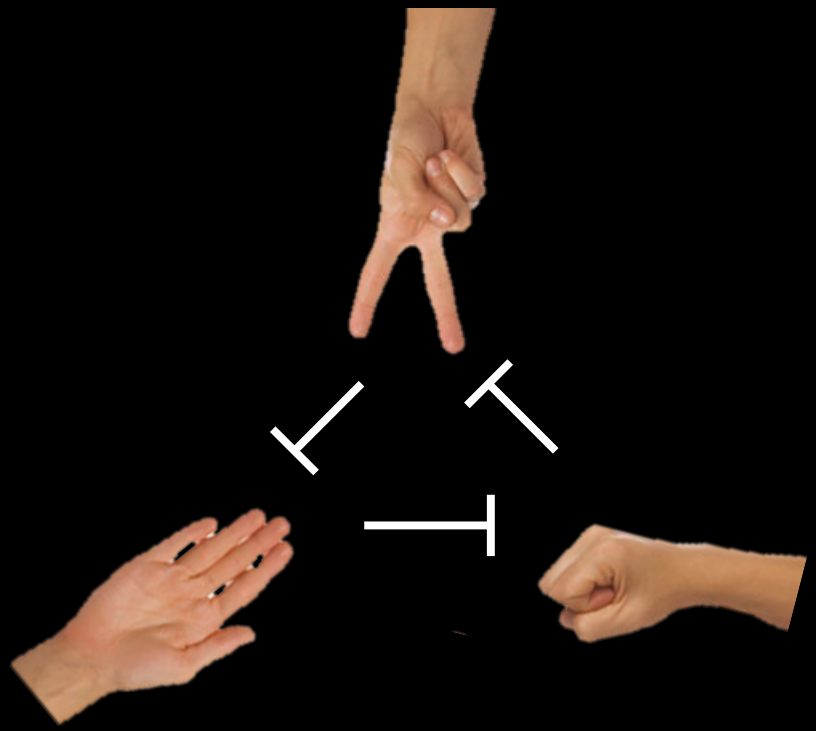
Drosophila circadian clock

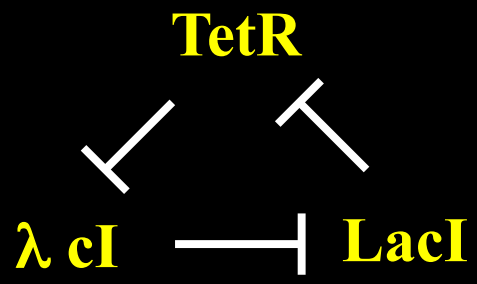
Is it possible to design a cellular clock?





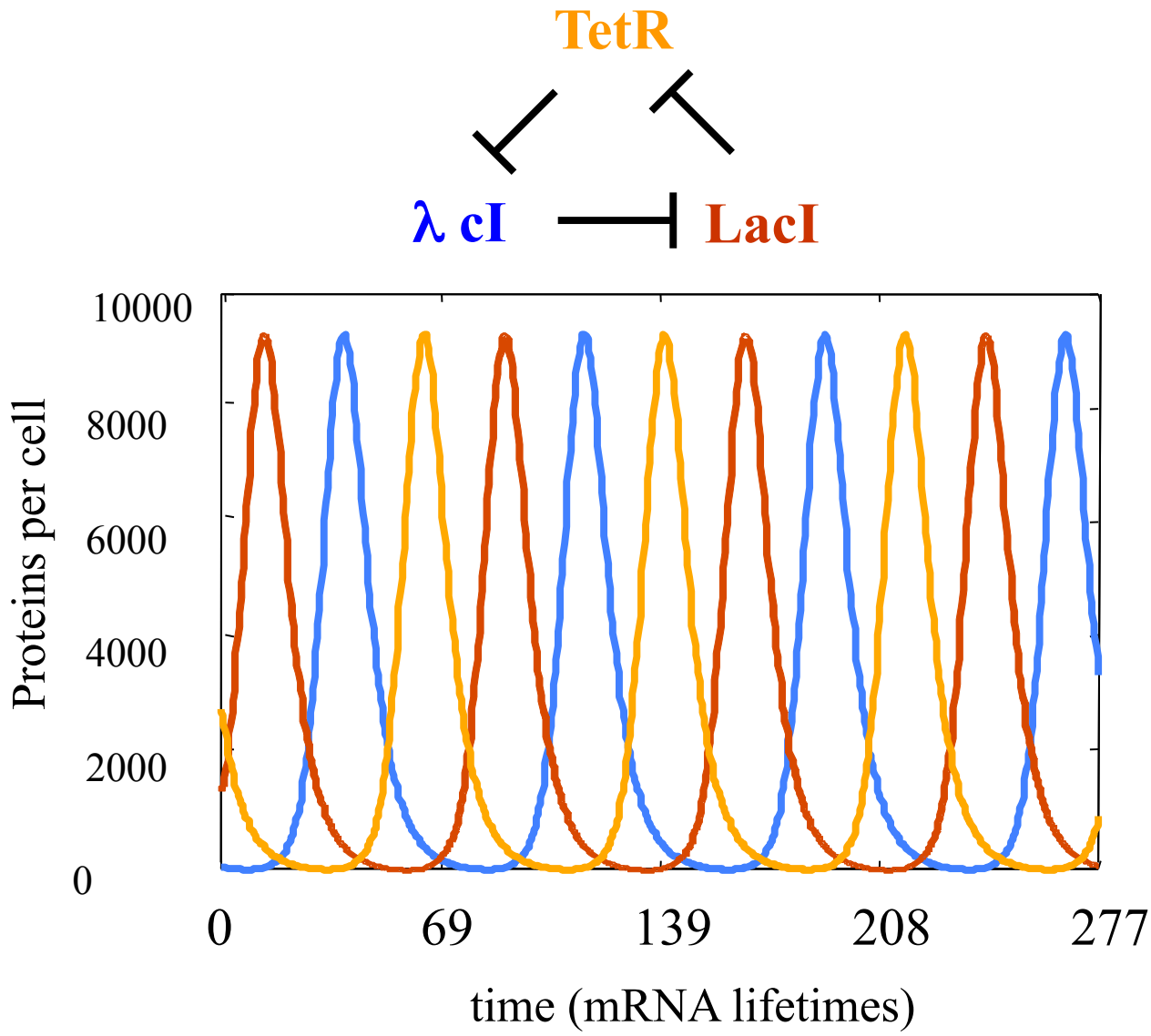
The Repressilator:
a synthetic genetic clock





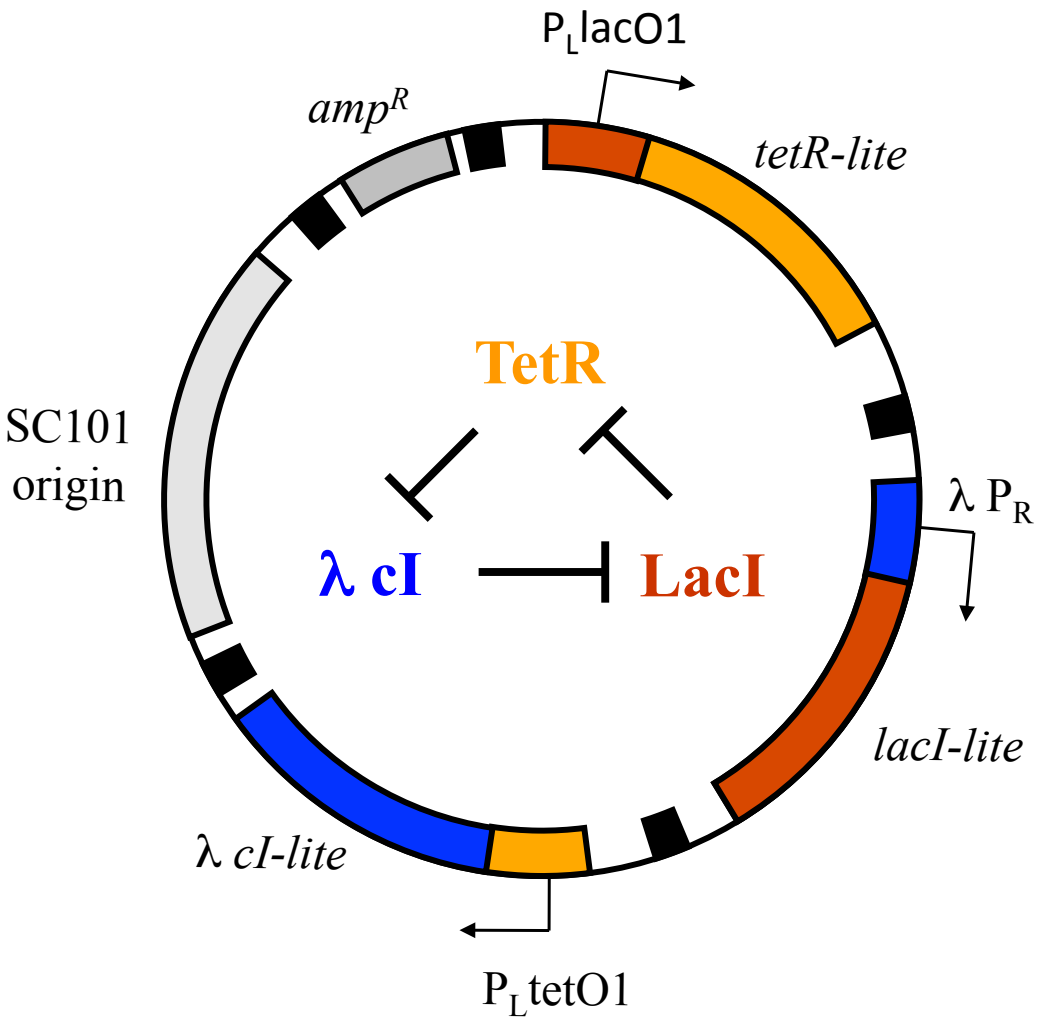
with Stanislas Leibler

Simulated *Repressilator* Oscillates

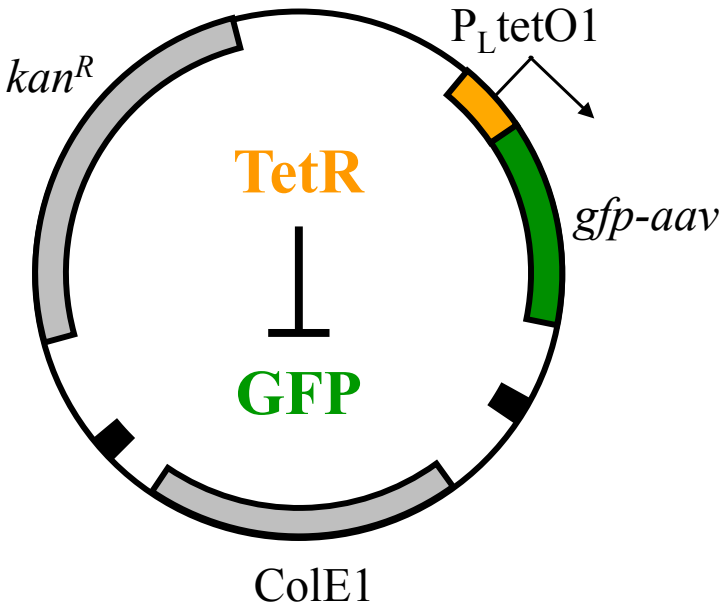


Plasmids

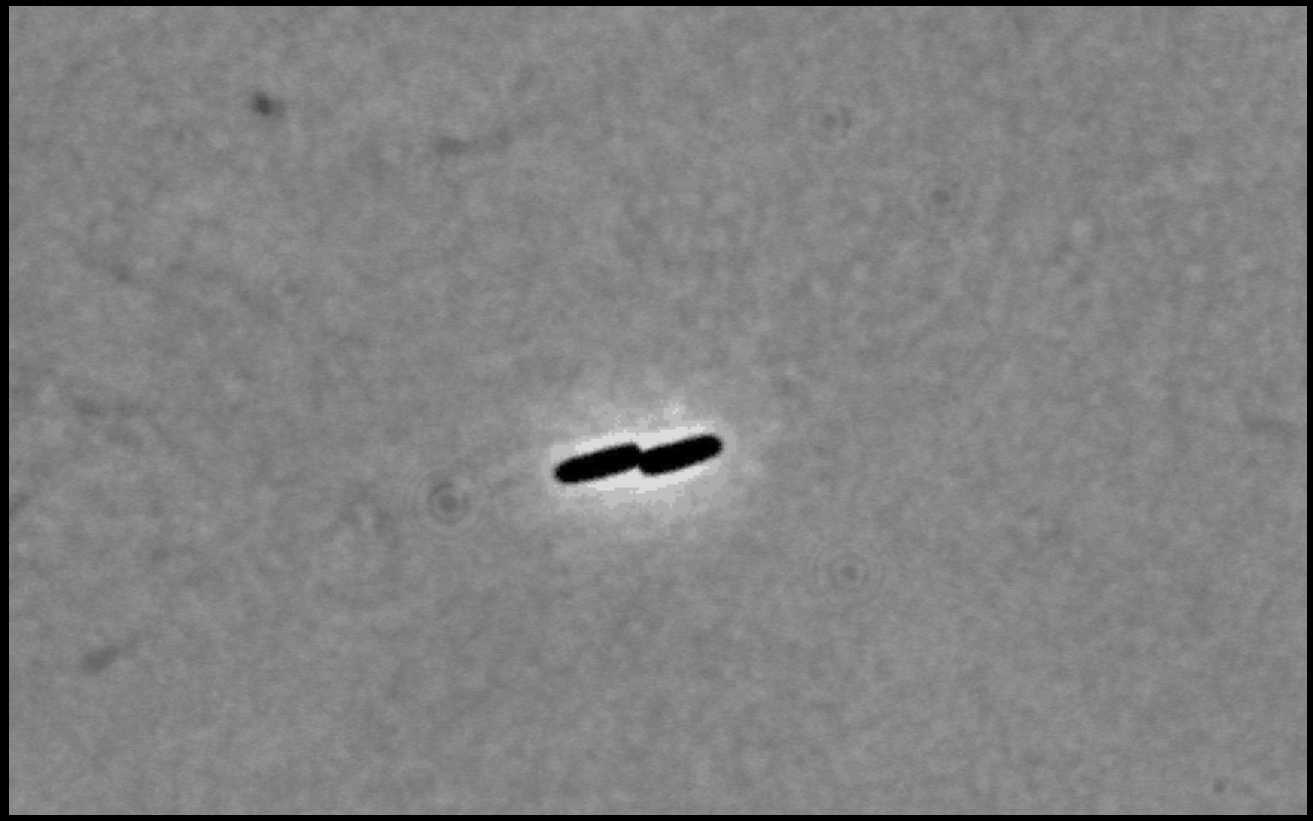
Repressilator



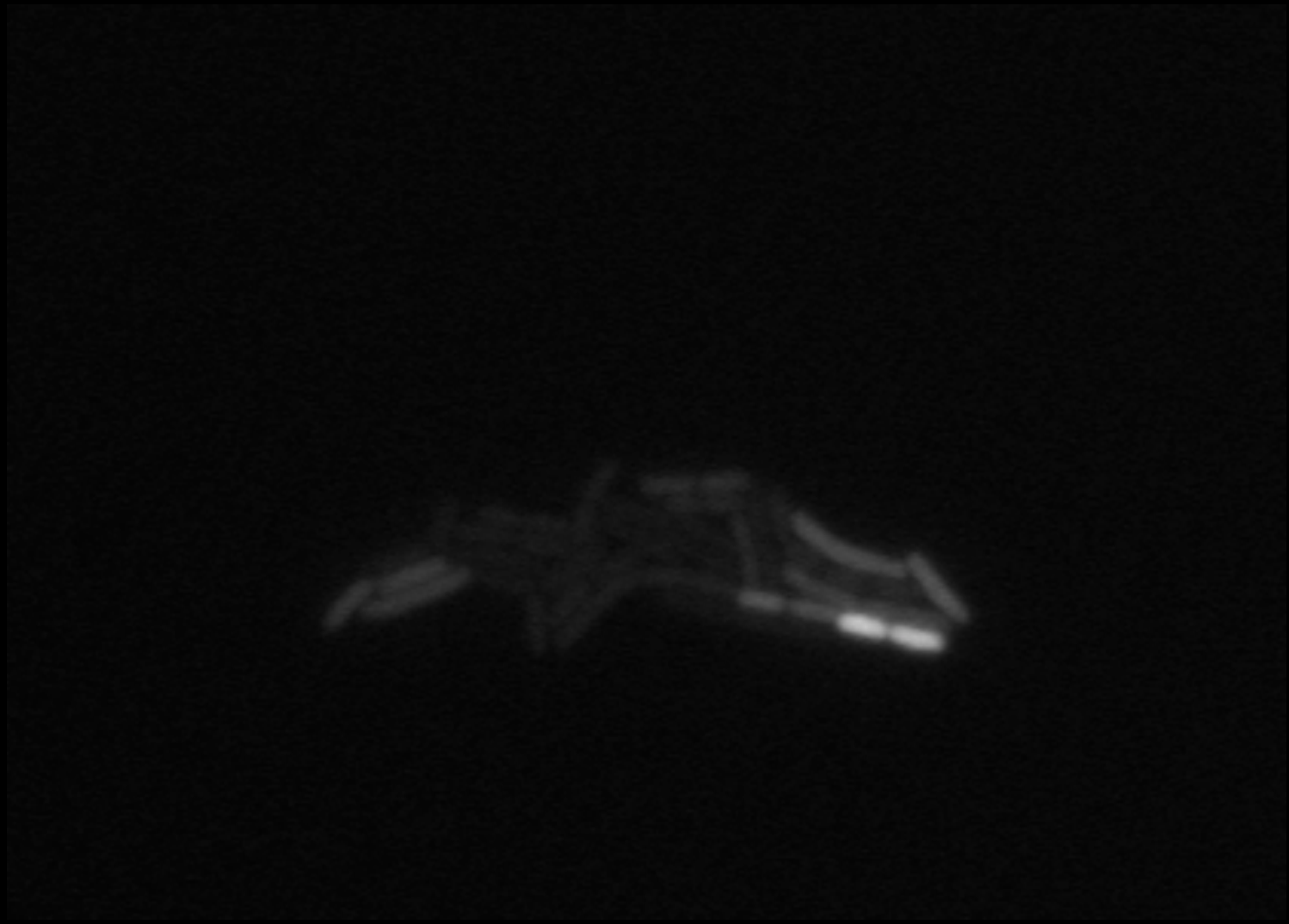
Reporter



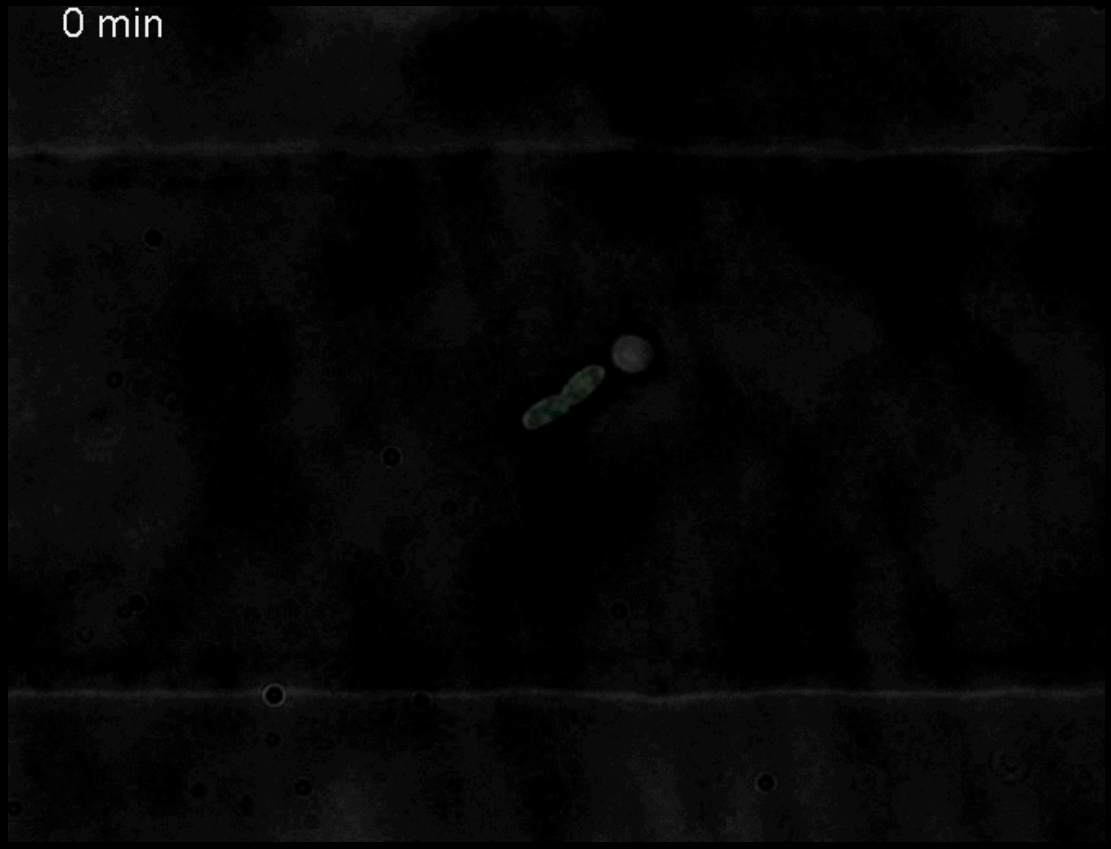
Repressilator movie



Repressilator movie



Improved Clocks



Stricker et al (Jeff Hasty lab, UCSD), *Nature* 2008

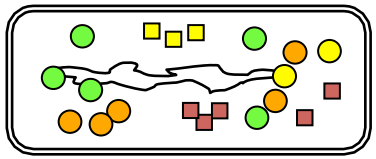
Repressilators and other synthetic circuits

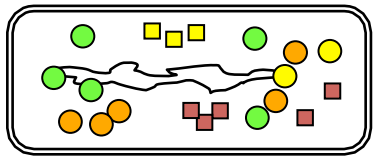
- Possible to design new behaviors in cells
- But where does the variability come from?

How deterministic is the cell?

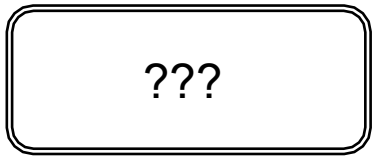
The thought experiment:

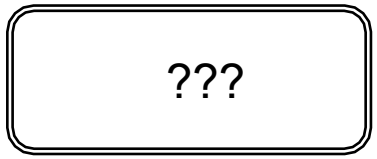
Now: two exactly identical cells



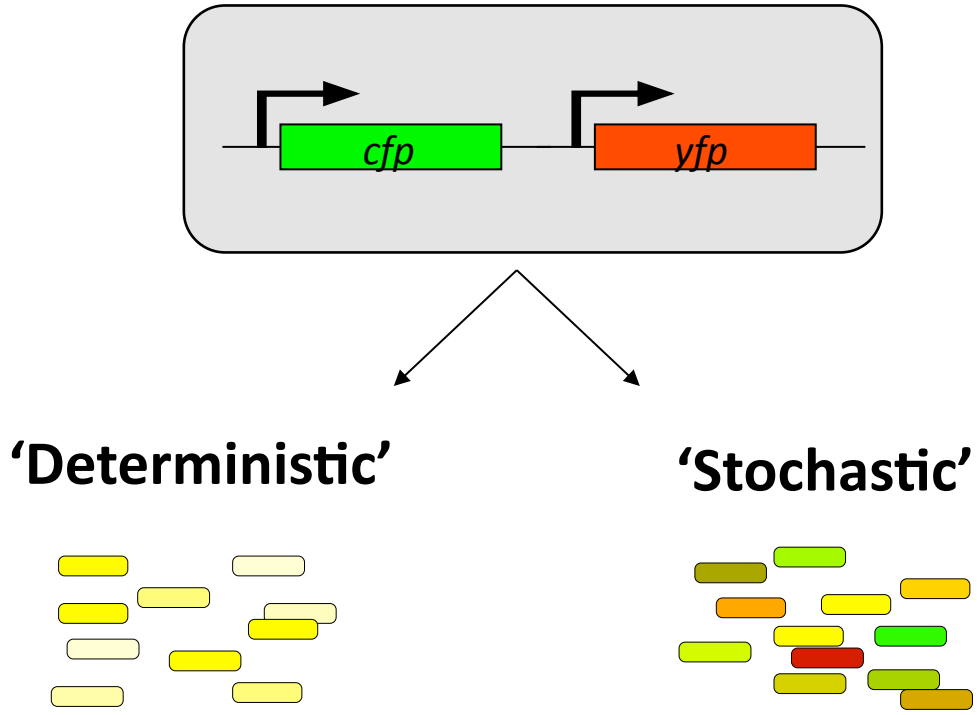


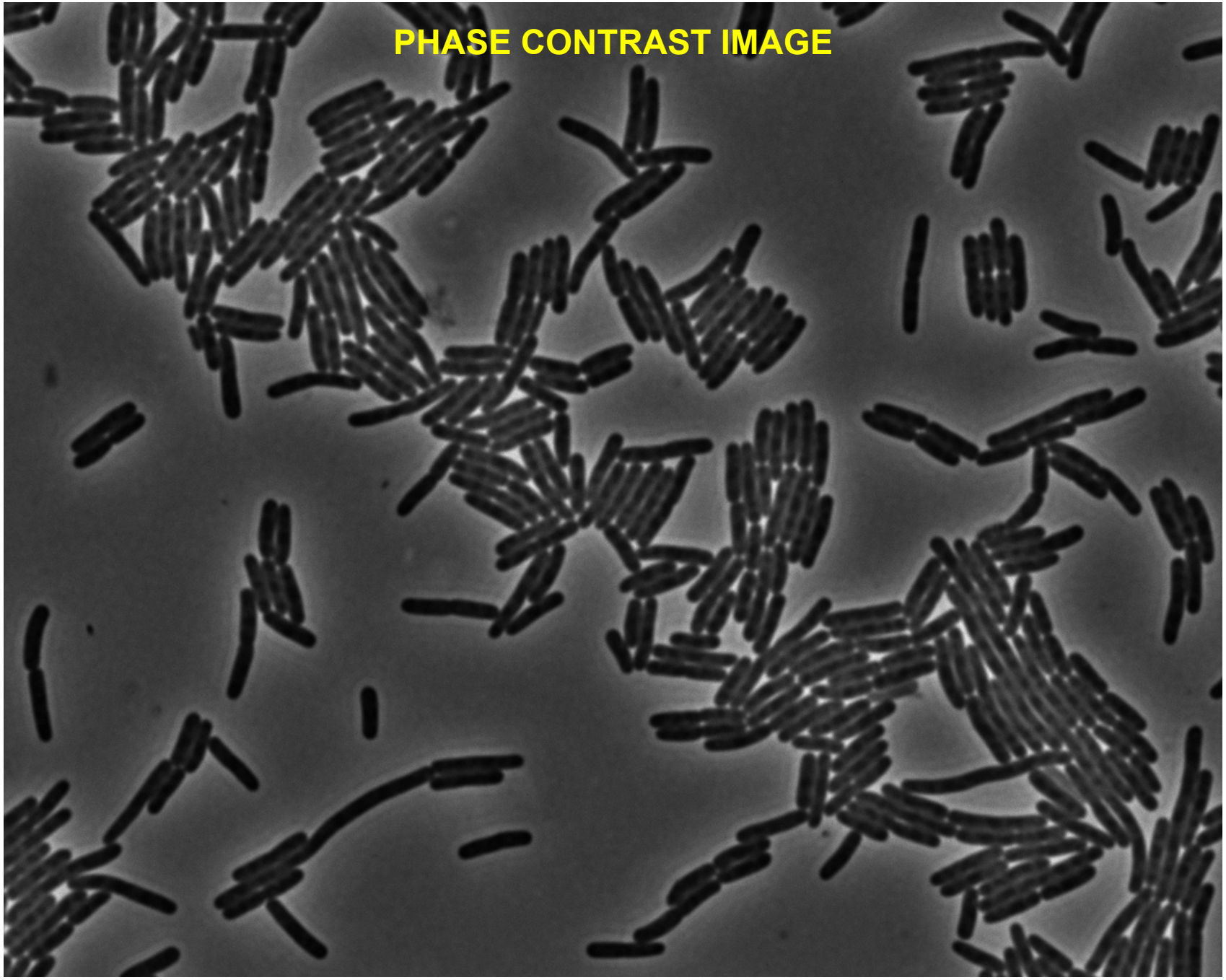
Later: still identical?

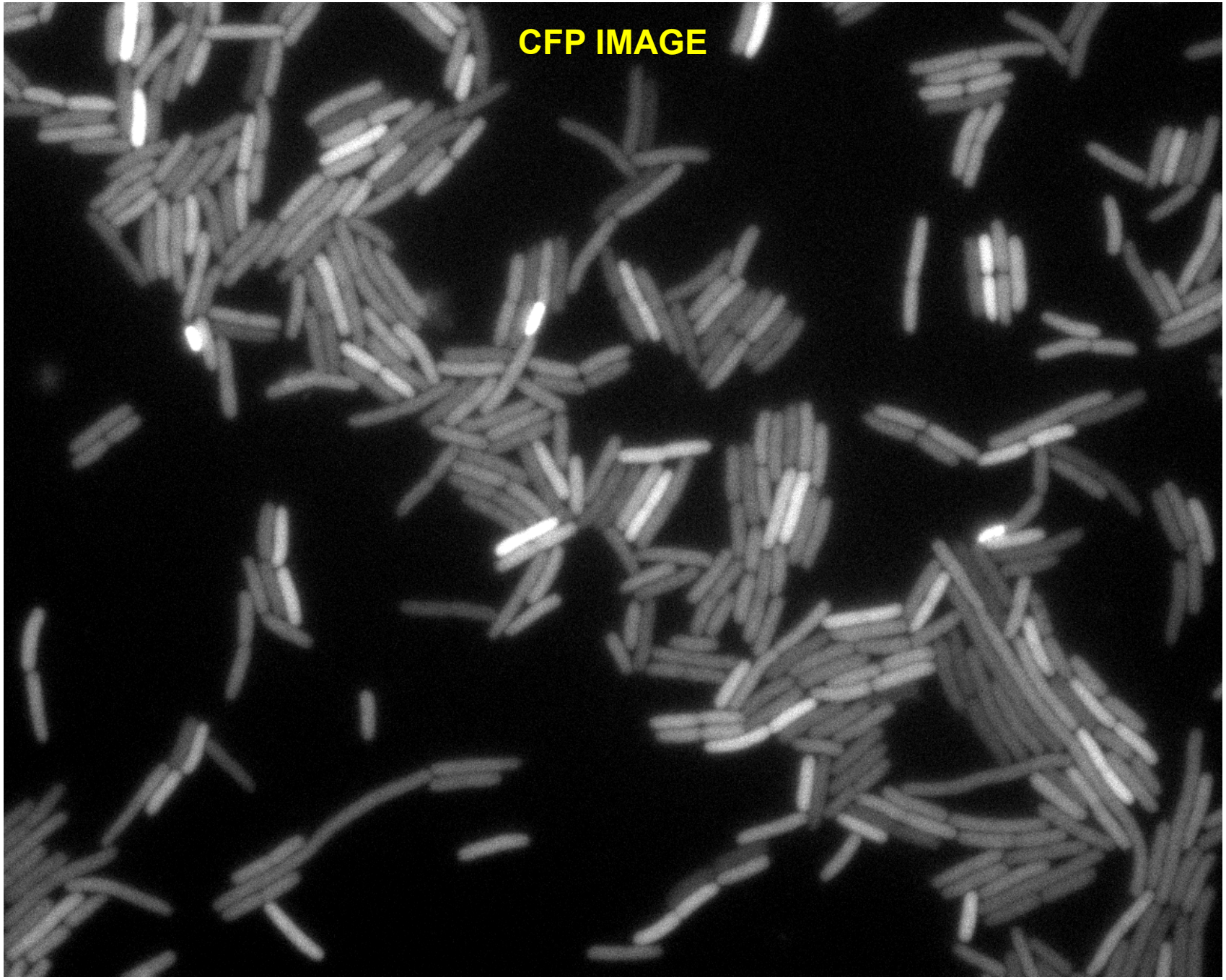


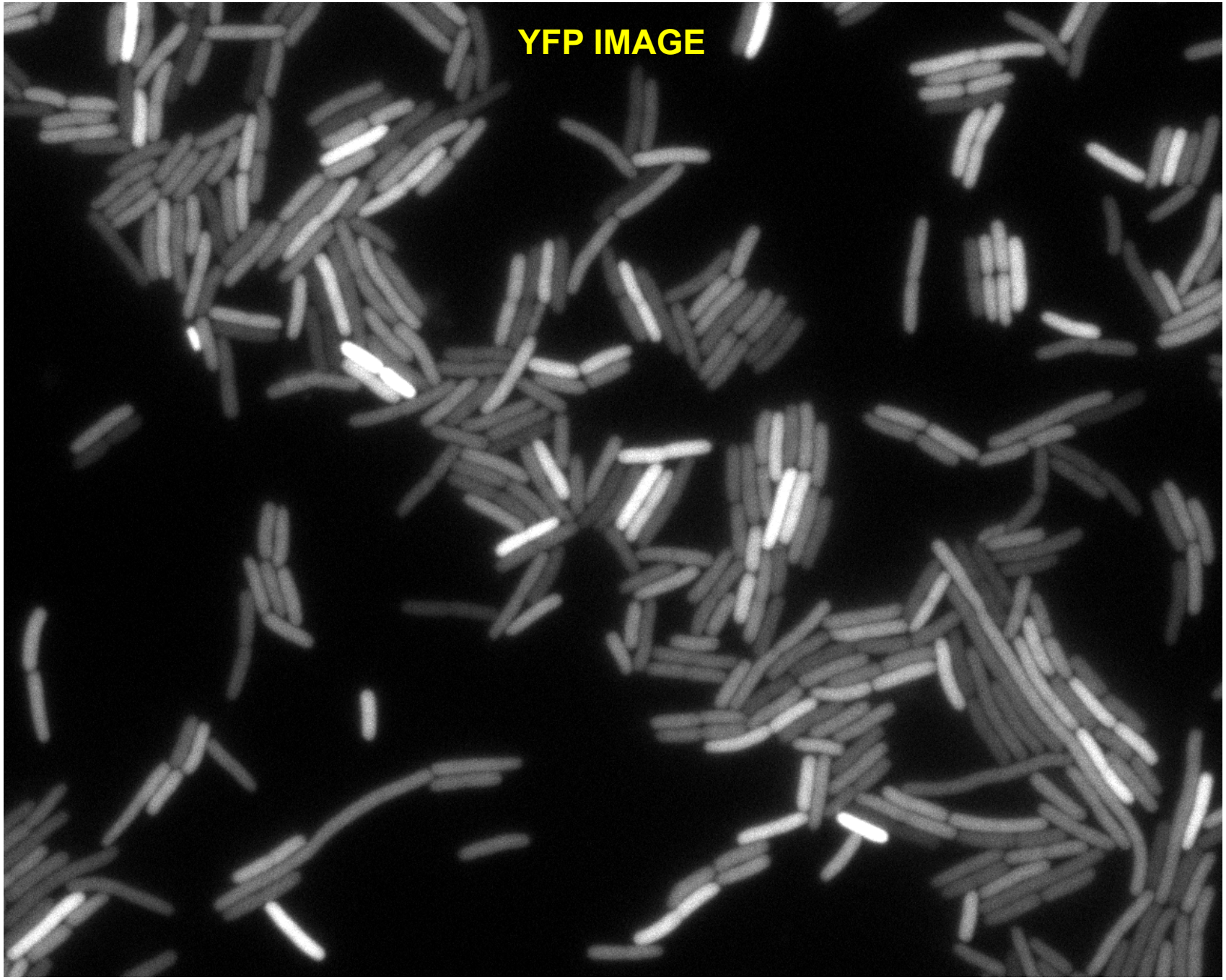


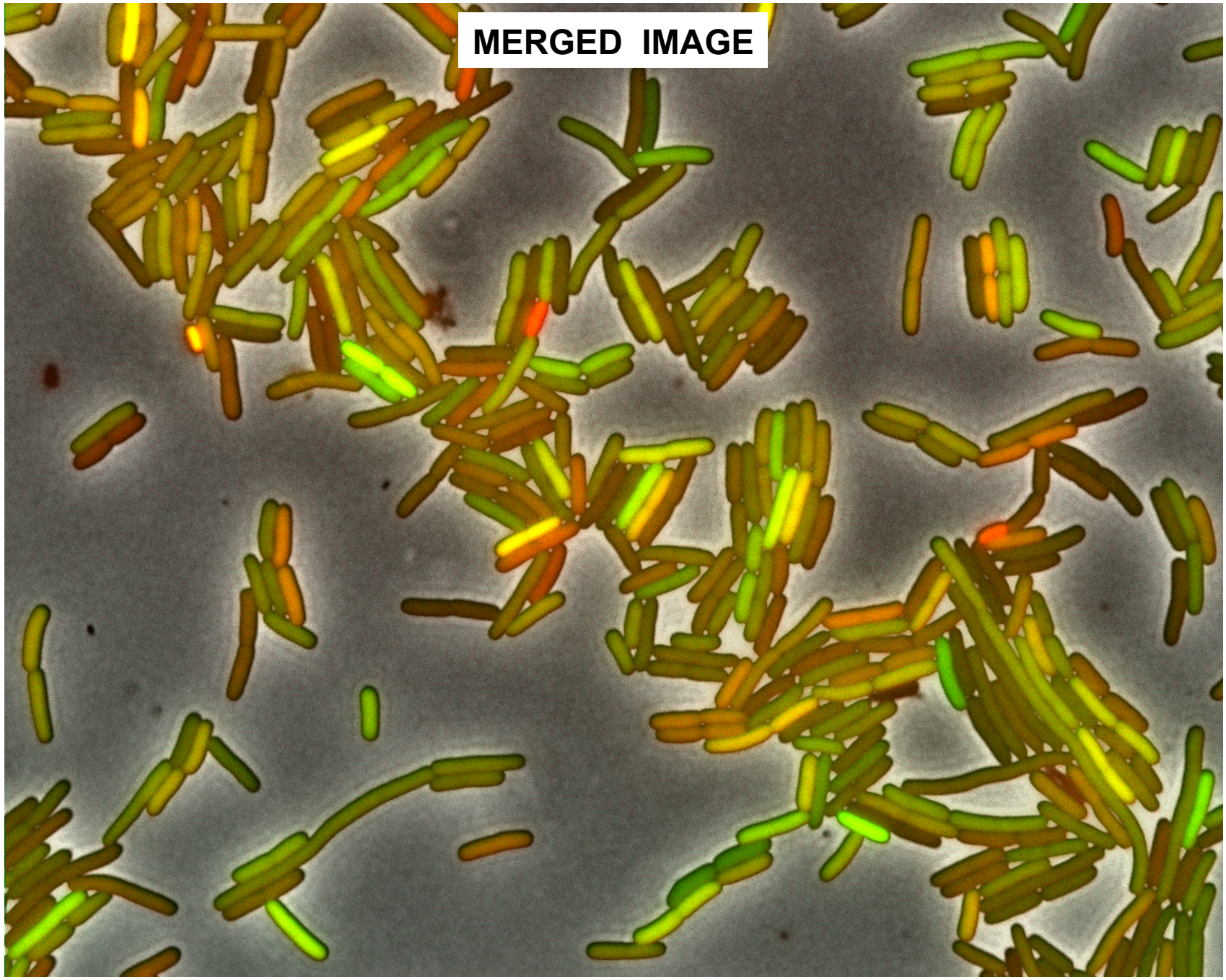
Origins of Variability
Are genetic circuits deterministic?




PHASE CONTRAST IMAGE


CFP IMAGE

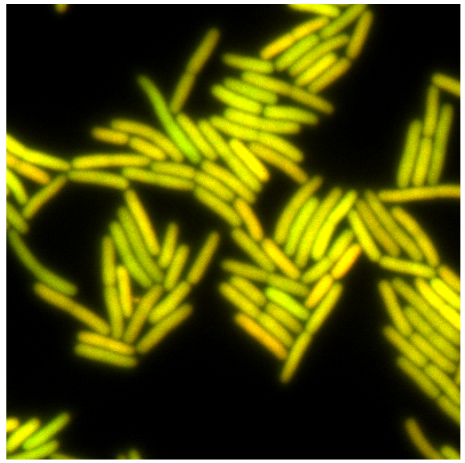

YFP IMAGE


MERGED IMAGE

Expression level controls noise level

"Quiet"

WT $_{(RPR37)}$ + IPTG

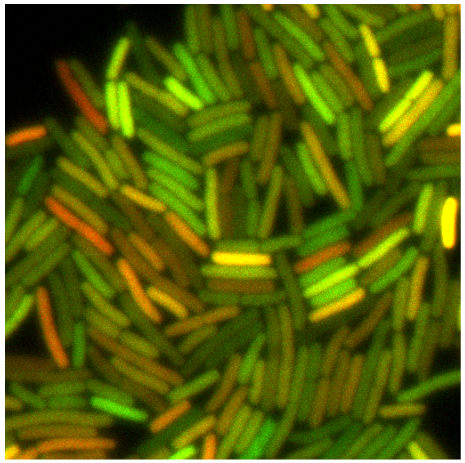


Intensity	= 1
η_{int}	= 0.063 (0.058-0.069)
η_{ext}	= 0.098 (0.09-1.1)

Decrease Expression 30-fold

"Noisy"

WT $_{(RPR37)}$



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

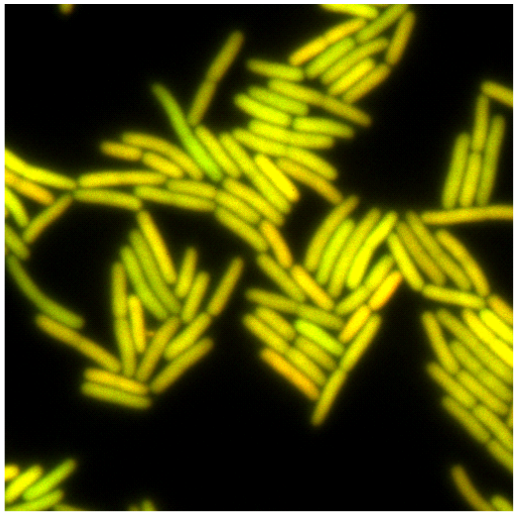
yfp

cfp

Noise is evolvable

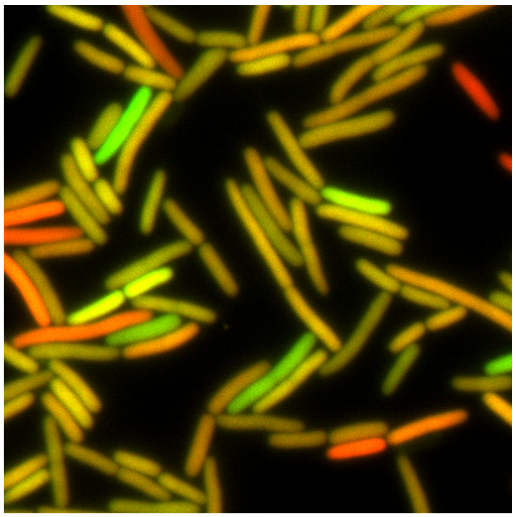
CFP=Green
YFP=Red

$WT_{(RPR37)}$+IPTG



Delete *recA* gene

$WT_{(RPR37)}$D*recA*+IPTG



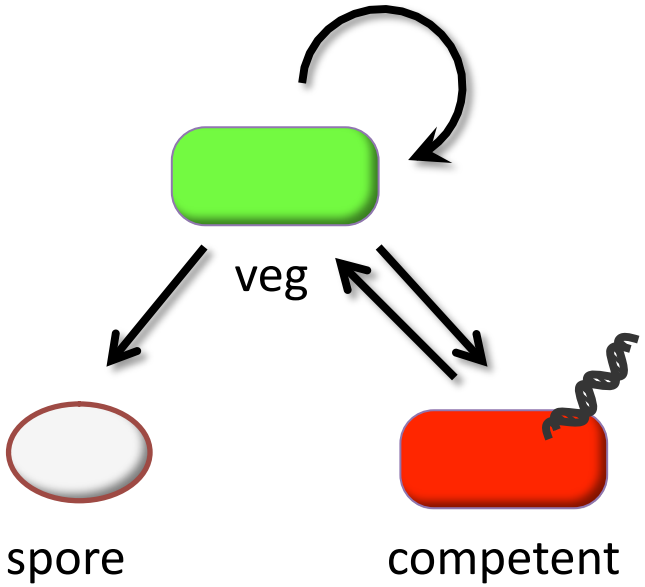
Intensity	= 1
h_{int}	= 0.063 (0.058-0.069)
h_{ext}	= 0.098 (0.09-1.1)

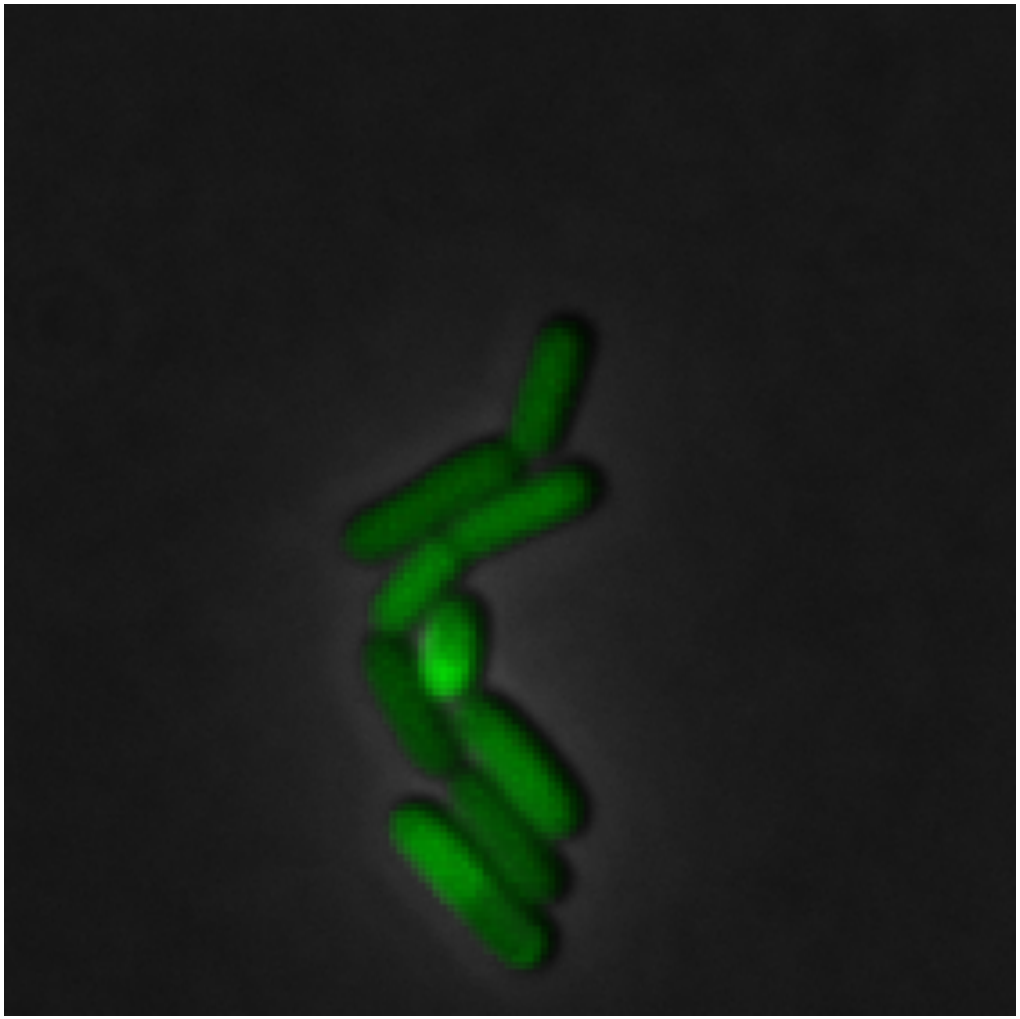
Intensity	= 1.2
h_{int}	= 0.17 (0.15-0.2)
h_{ext}	= 0.12 (0.088-0.14)

Noise is an evolvable trait

Noise:
More than a Nuisance?

Bacillus subtilis differentiates probabilistically and transiently

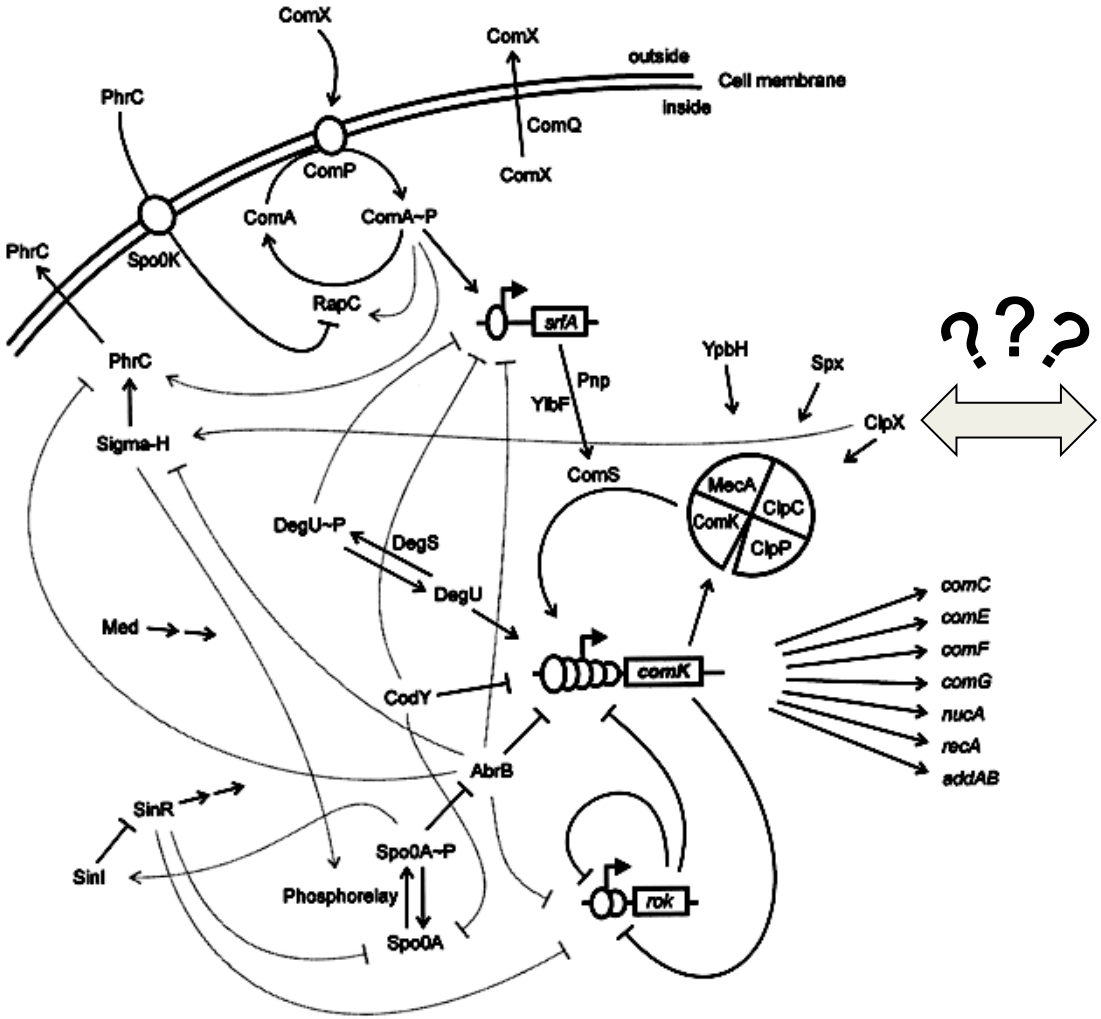


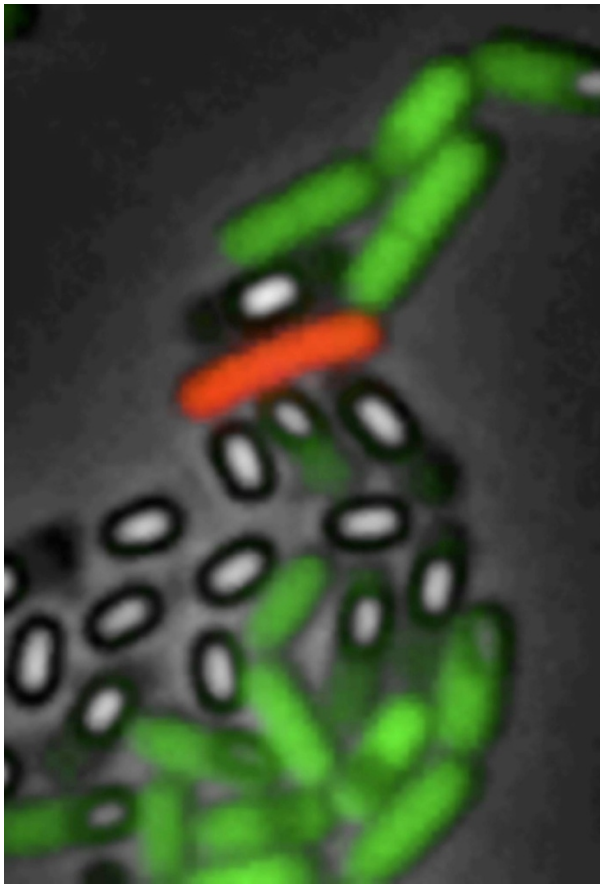


Bacillus subtilis

G. Suel et al, Nature 2006,
Science 2007; Catagay et al, Cell 2009

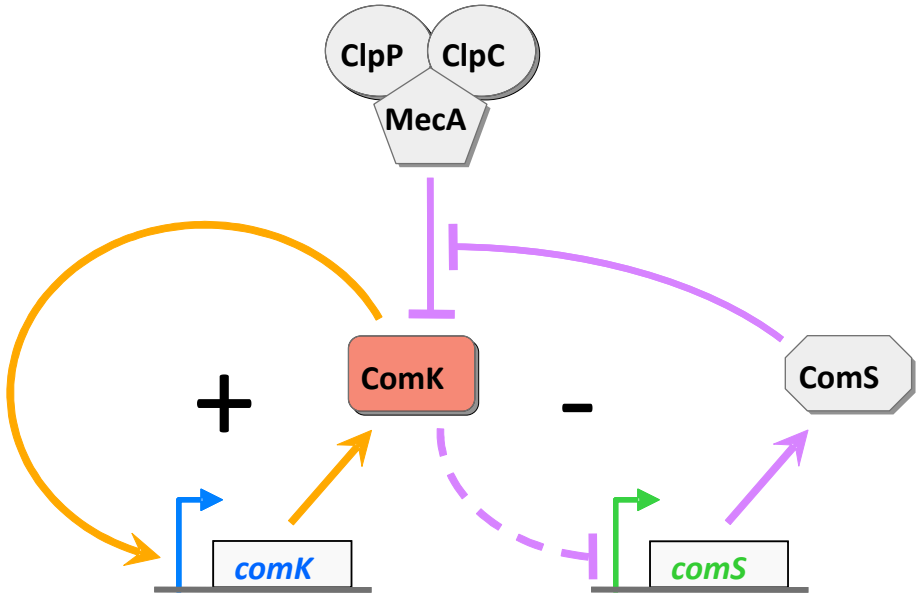
The Genetic Circuitry of Competence





Competence circuit diagram from Hamoen et al. *Microbiology*. 2003

A relatively simple core feedback circuit controls competence differentiation



Combination of fast positive and slow negative feedback loops

Mathematical Model of the Circuit

ComK-regulated expression

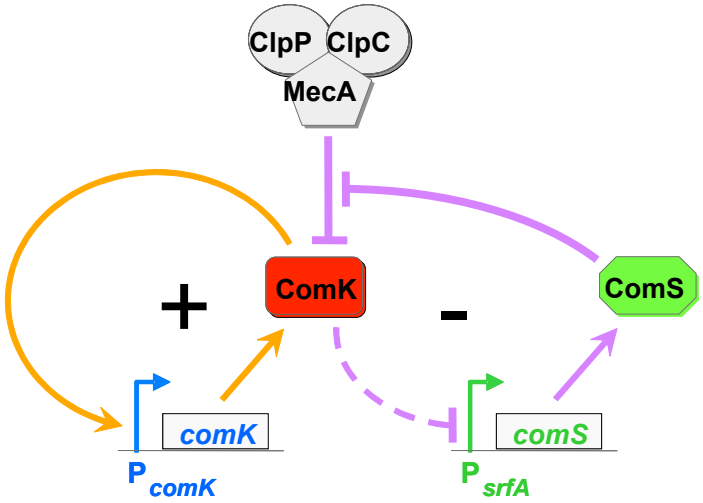
Basal degradation

Basal expression

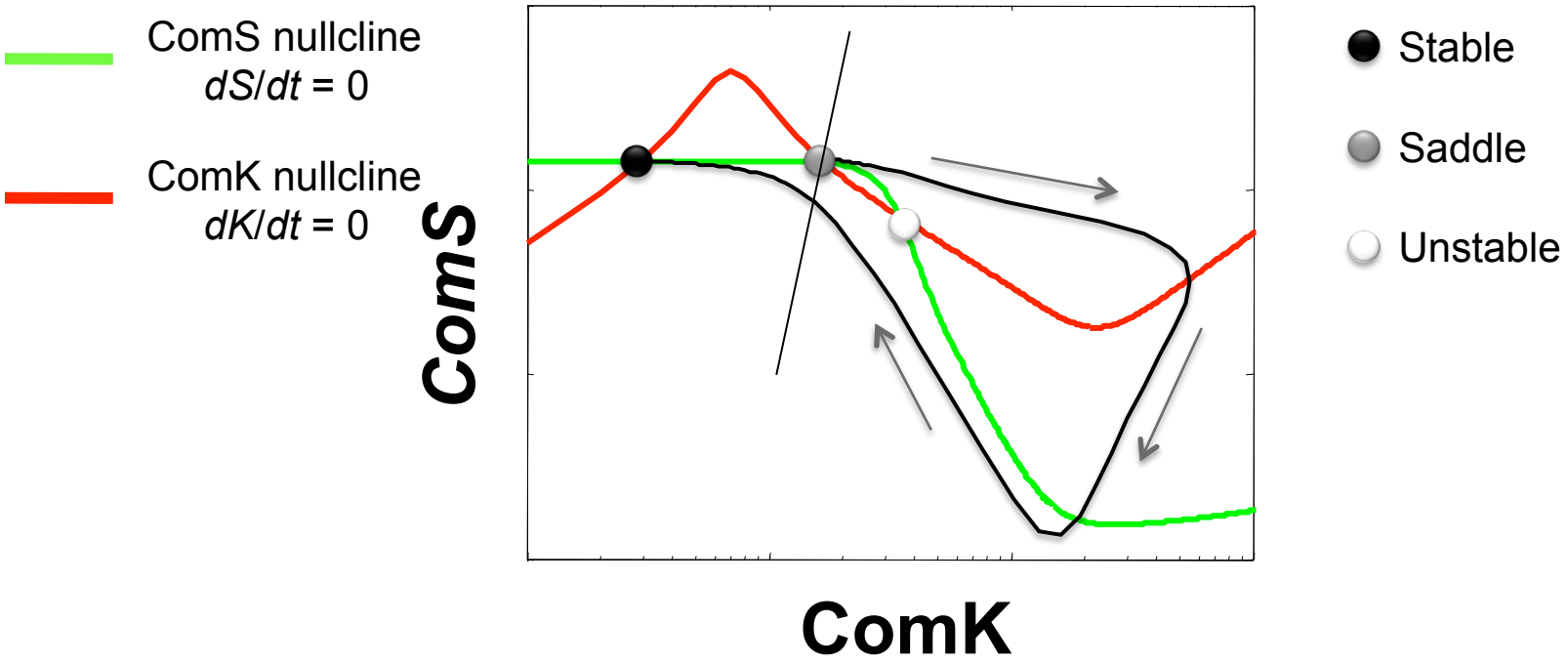
Degradation By MecA

$$\frac{dK}{dt} = a_k + \frac{b_k K^n}{k_0^n + K^n} - \frac{K}{1+K+S} - \Delta_k K$$

$$\frac{dS}{dt} = a_s + \frac{b_s}{1+(K/k_1)^p} - \frac{S}{1+K+S} - \Delta_s S$$

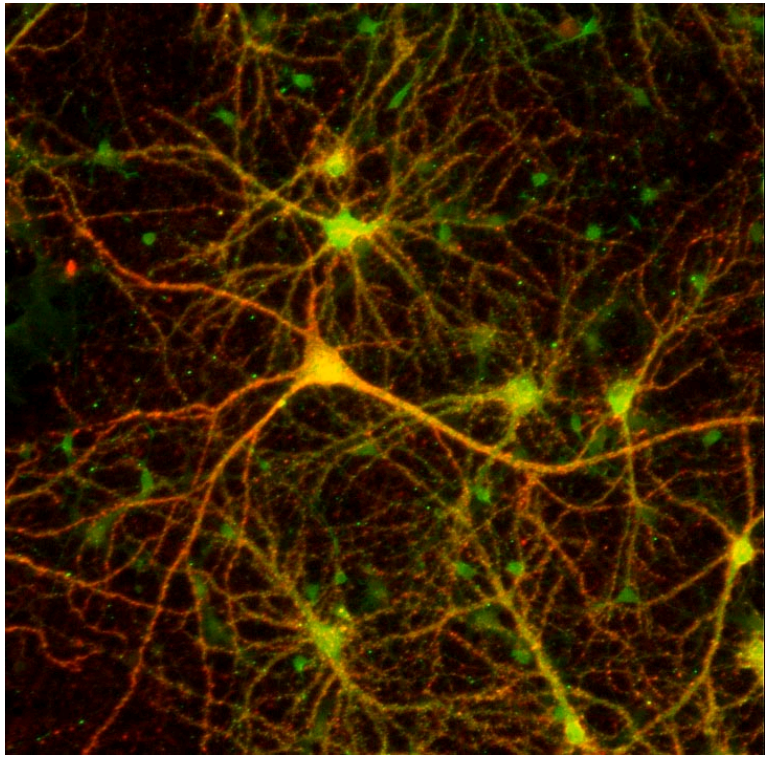


Excitability: A Design Principle for Probabilistic, Transient Differentiation



Other excitable systems in biology

Neuron (Action Potential)

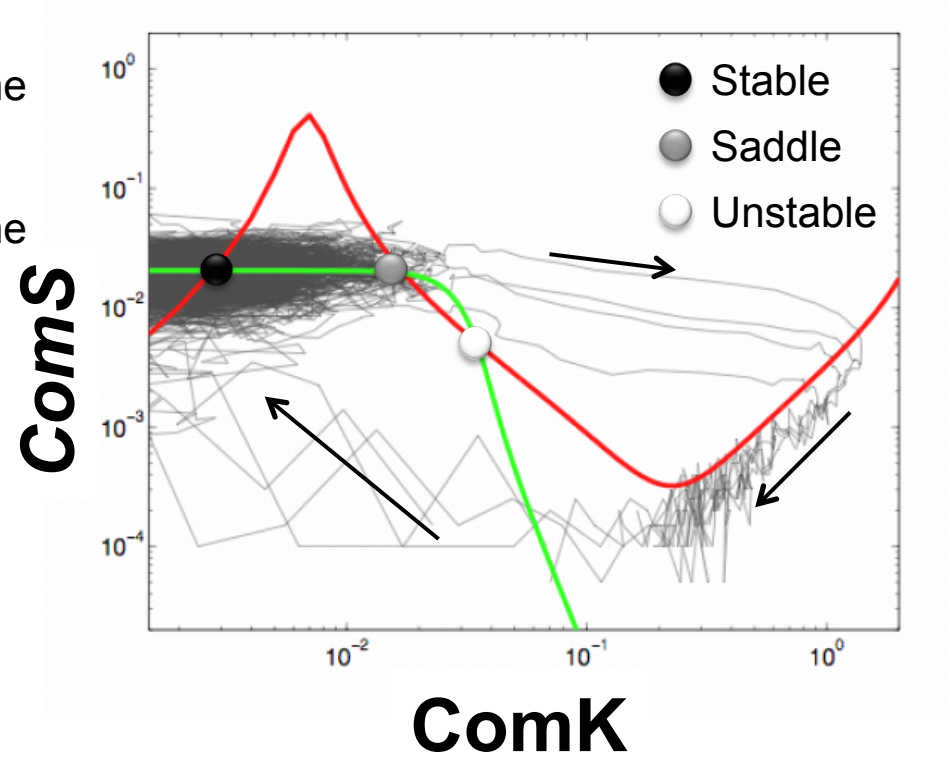


Toilet (Flush)

Hypothesis: Noise (fluctuations) cause differentiation

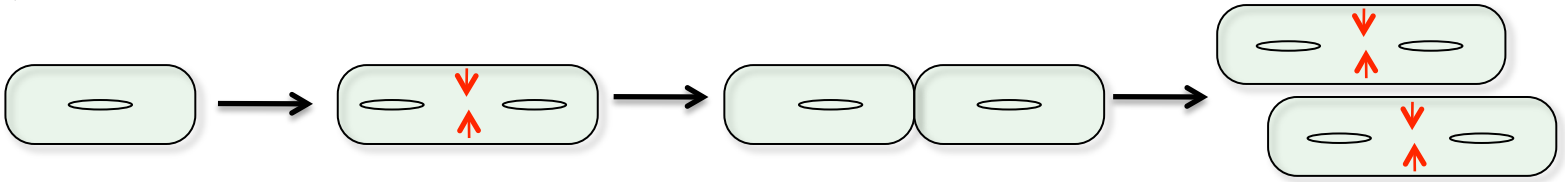
ComS nullcline
$dS/dt = 0$

ComK nullcline
$dK/dt = 0$

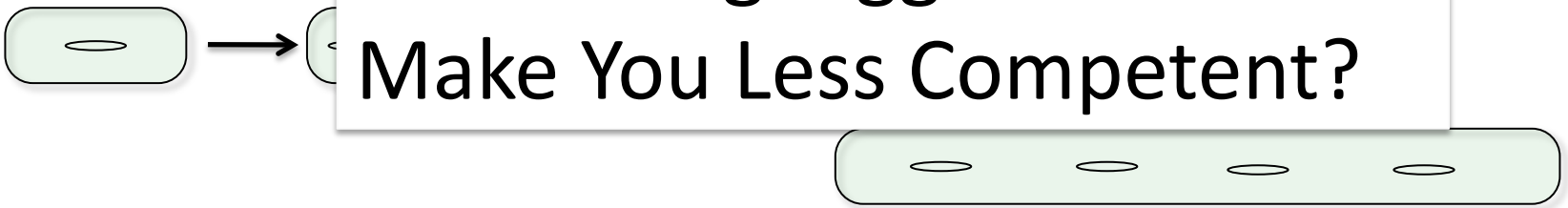


Filaments: a simple way to reduce cellular noise

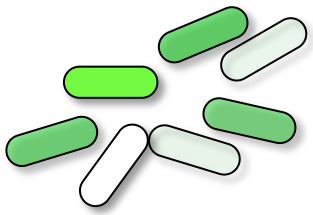
Normal cells:

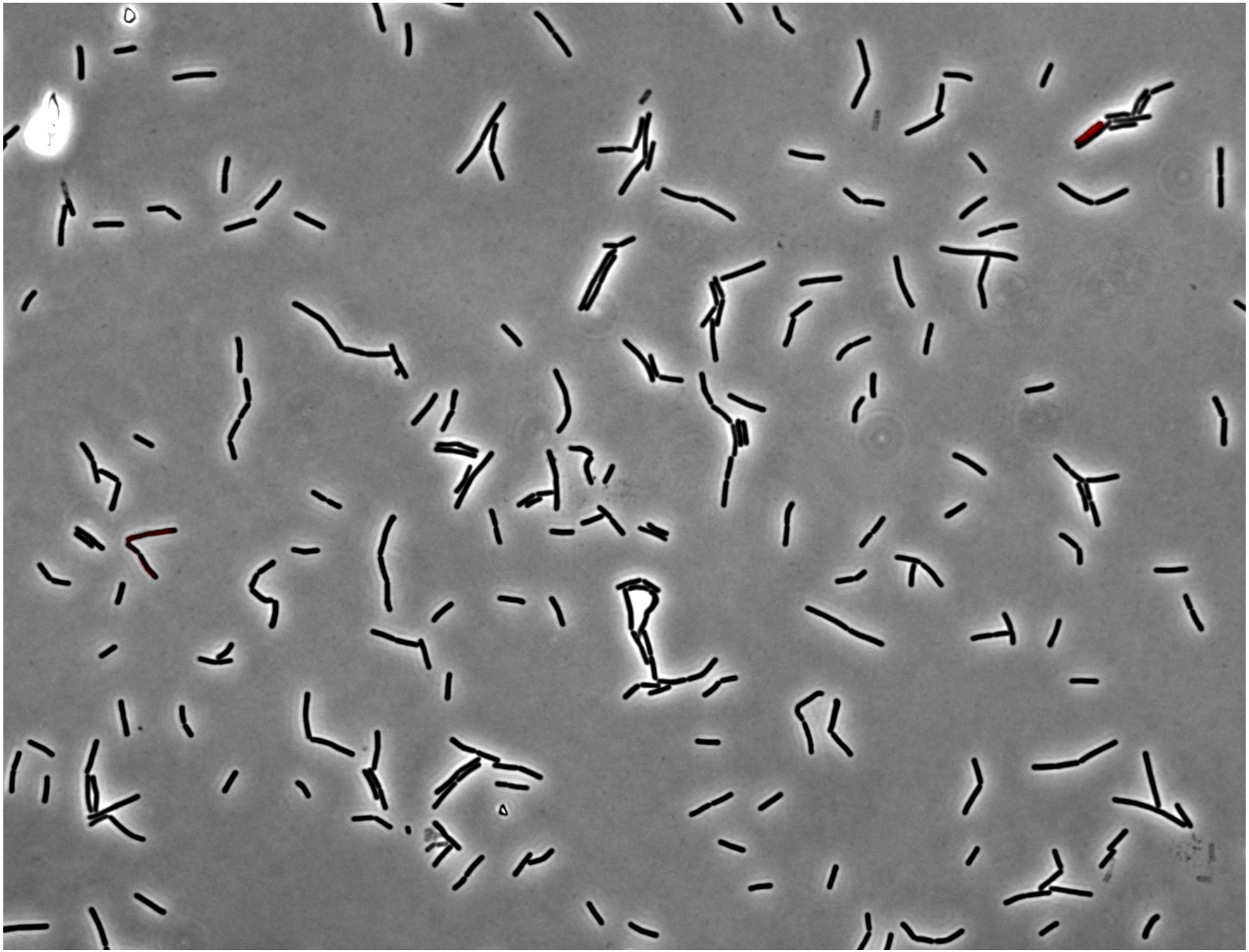


Filaments

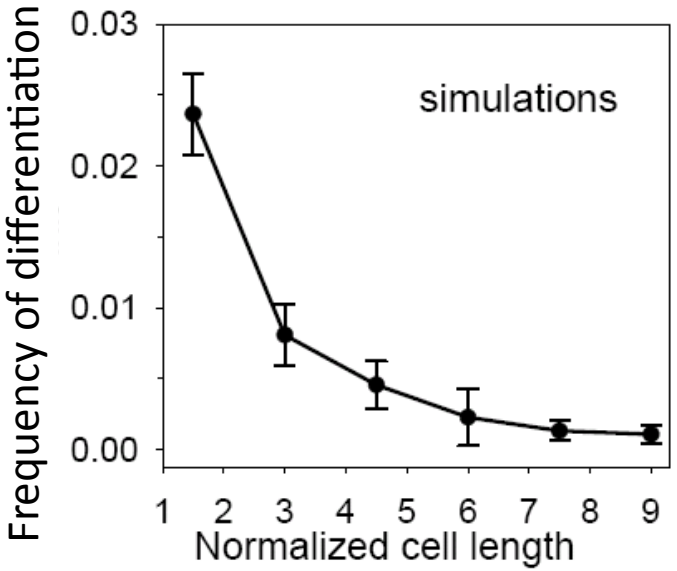


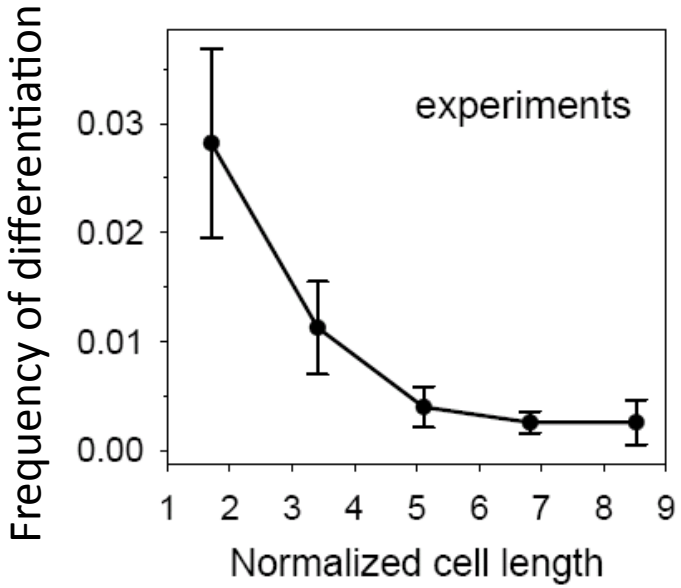
Does Being Bigger & Quieter Make You Less Competent?





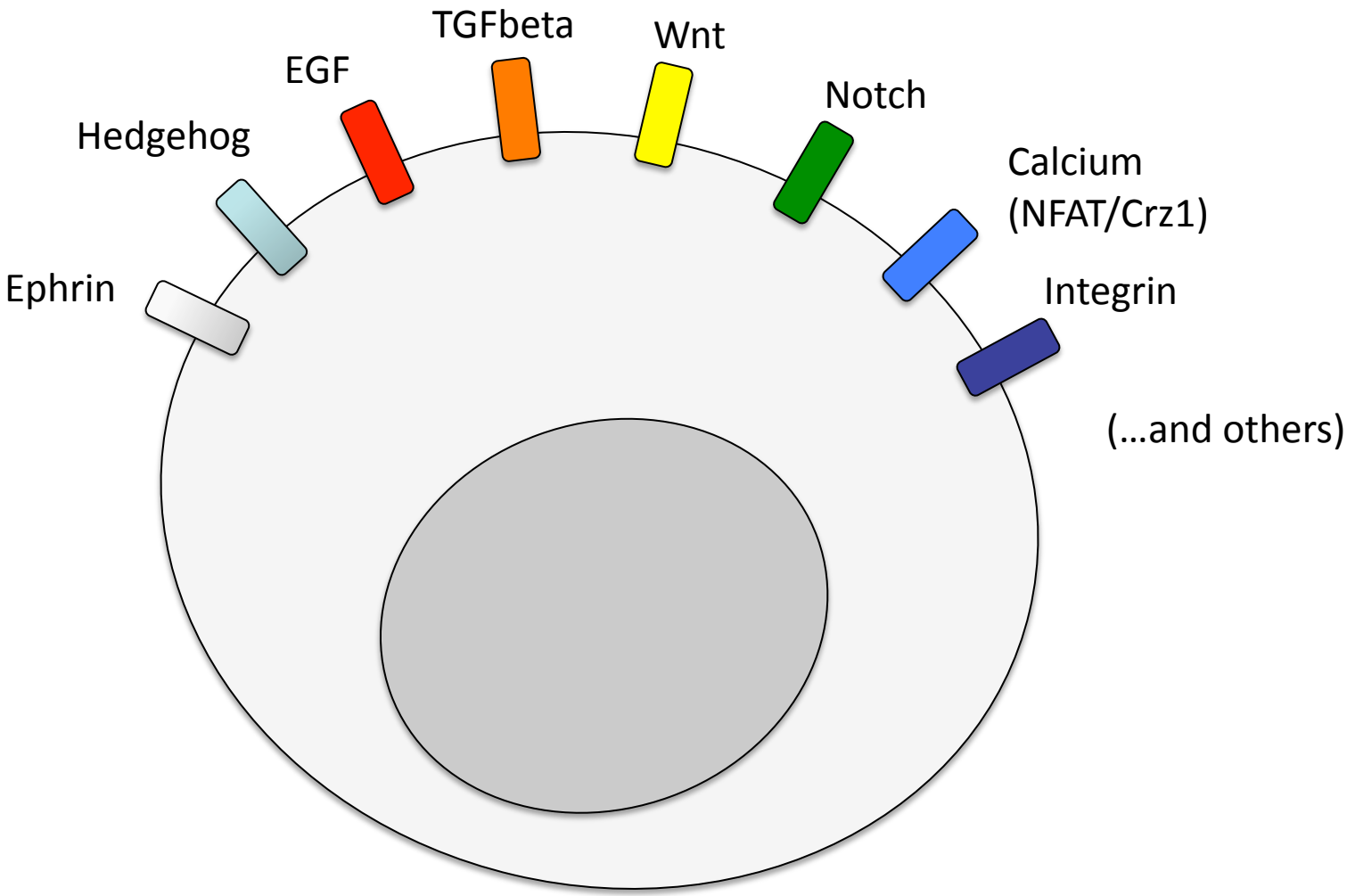
Longer cells fail to differentiate



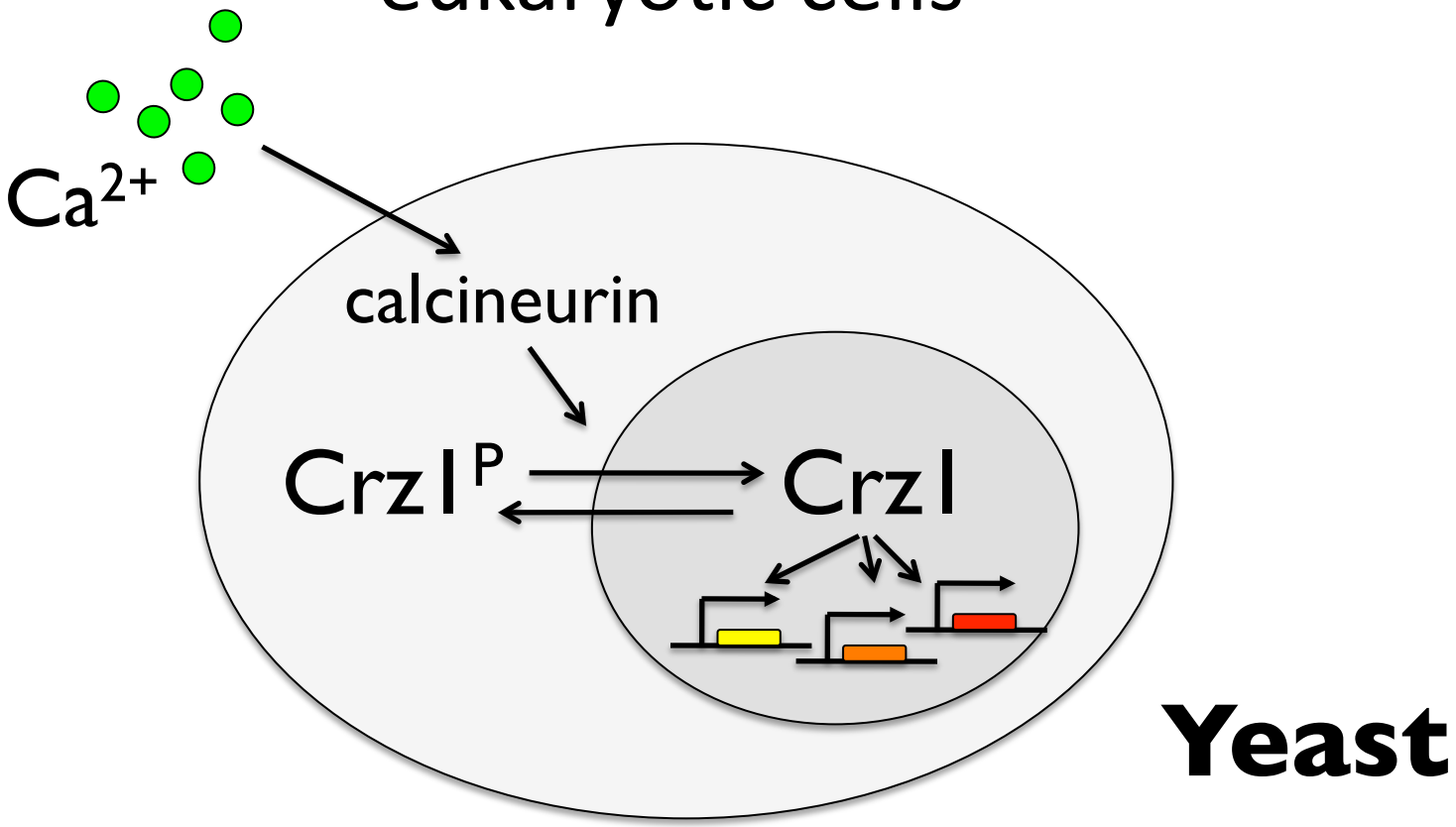


What else can noise do?

Cells Use Signaling Circuits to Communicate



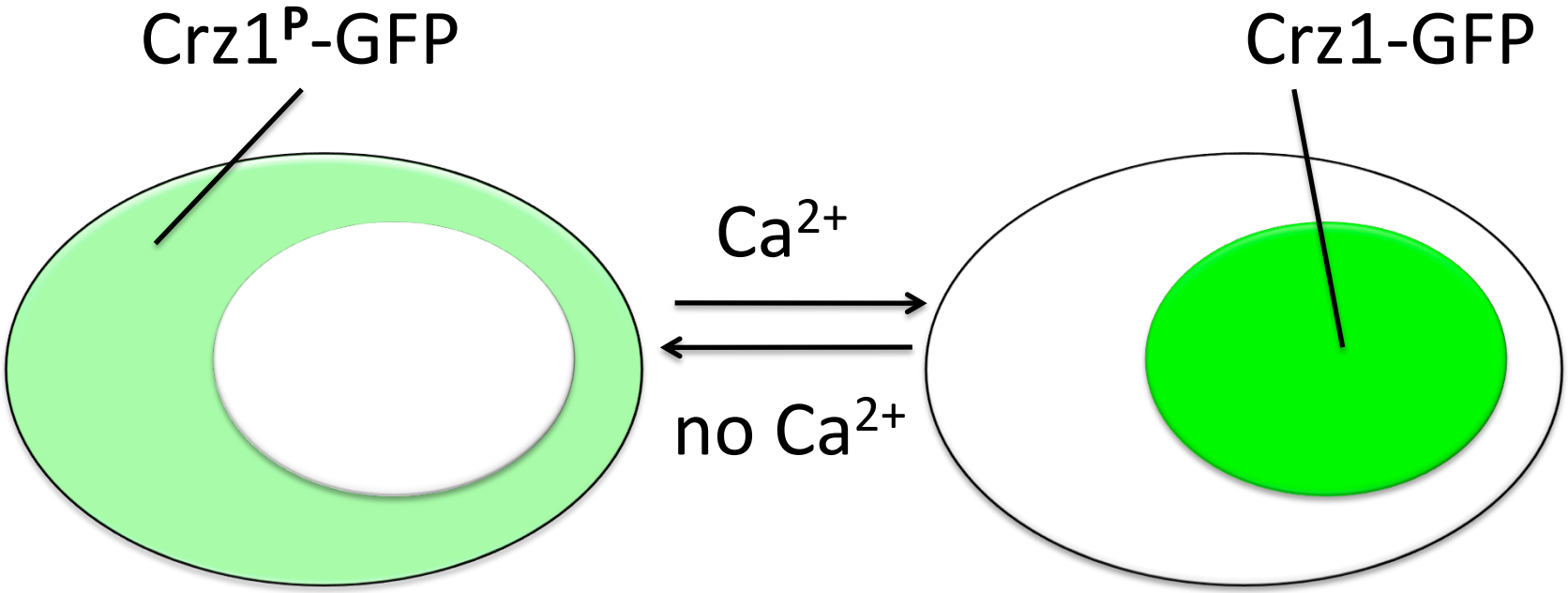
Ca^{2+} signaling regulates many processes in eukaryotic cells



Functionally similar to mammalian NF-AT.

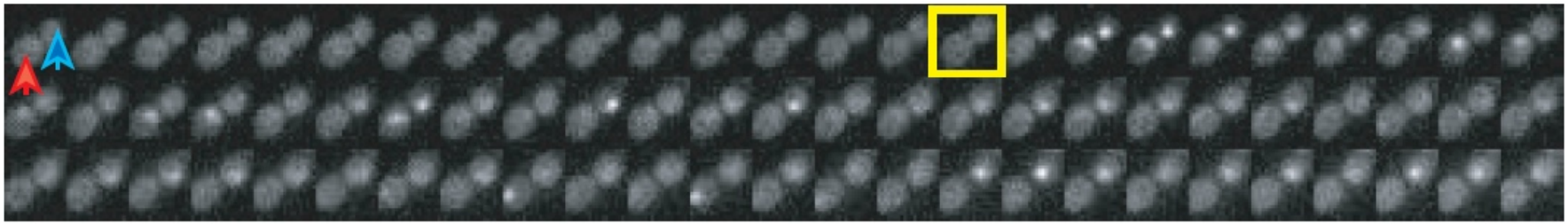
See also: Stathopoulos and Cyert, Genes Dev. 1997, 1999, Matheos et al, Genes Dev. 1997

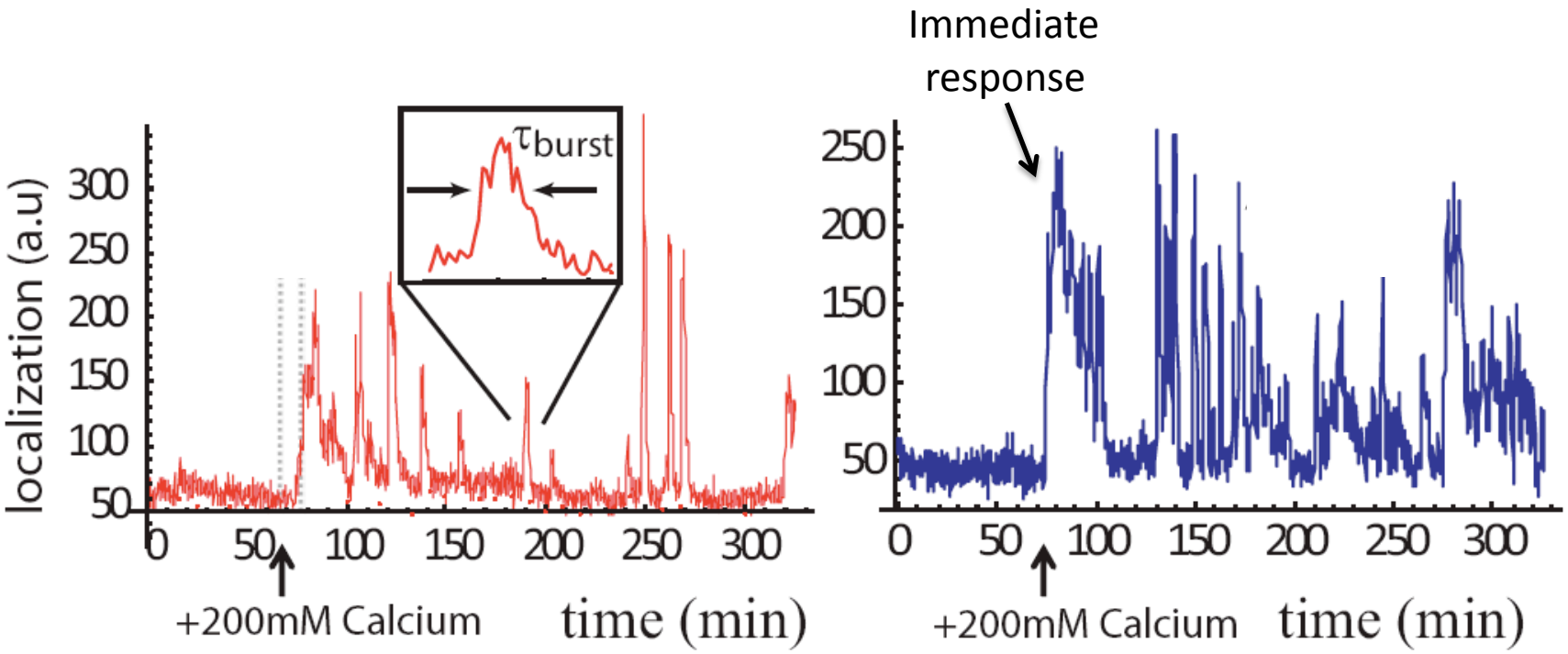
Crz1-GFP localization can be observed in individual cells



Movie of Crz1 After Ca^{2+} Addition

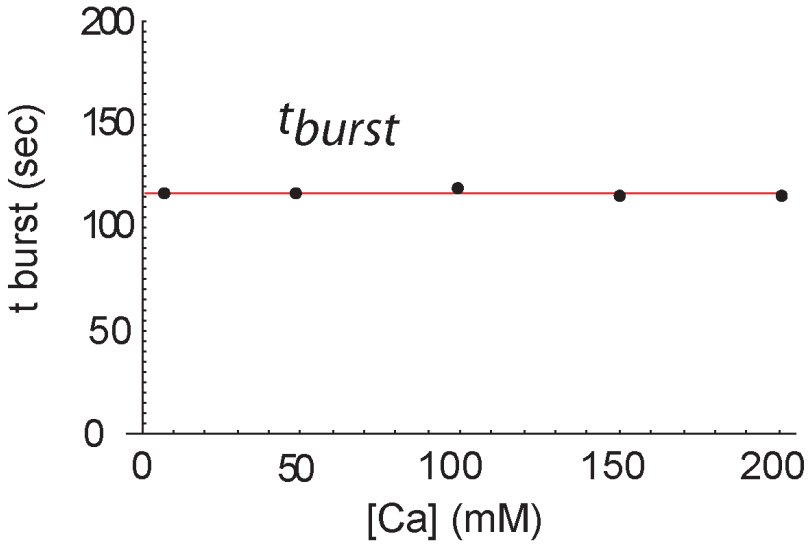
Crz1 Localizes in Stochastic Bursts



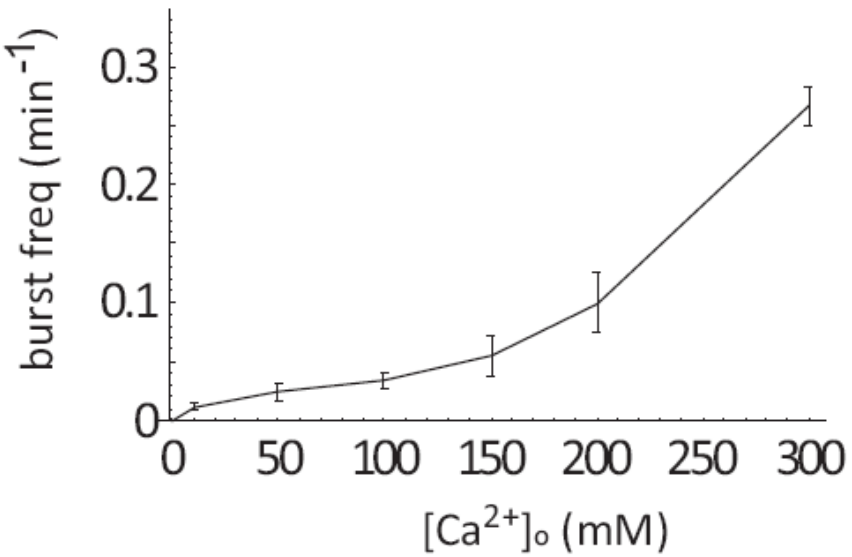


Calcium regulates the frequency, but not the duration, of bursts.

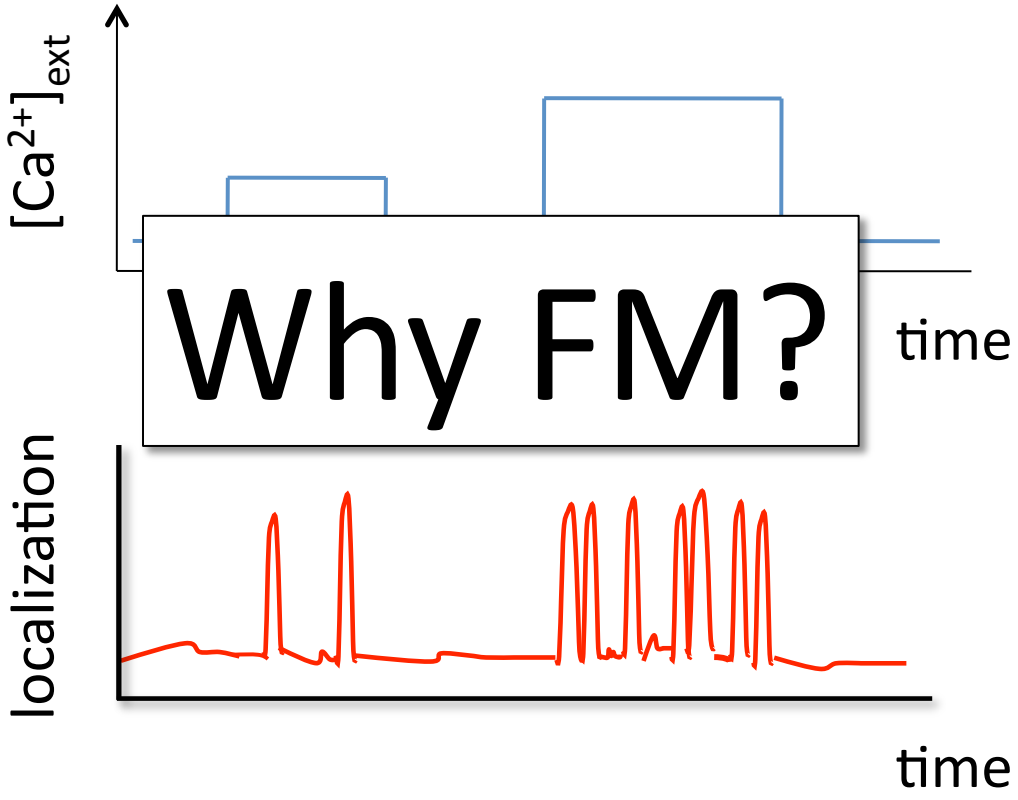
Mean Burst Duration



Burst Frequency



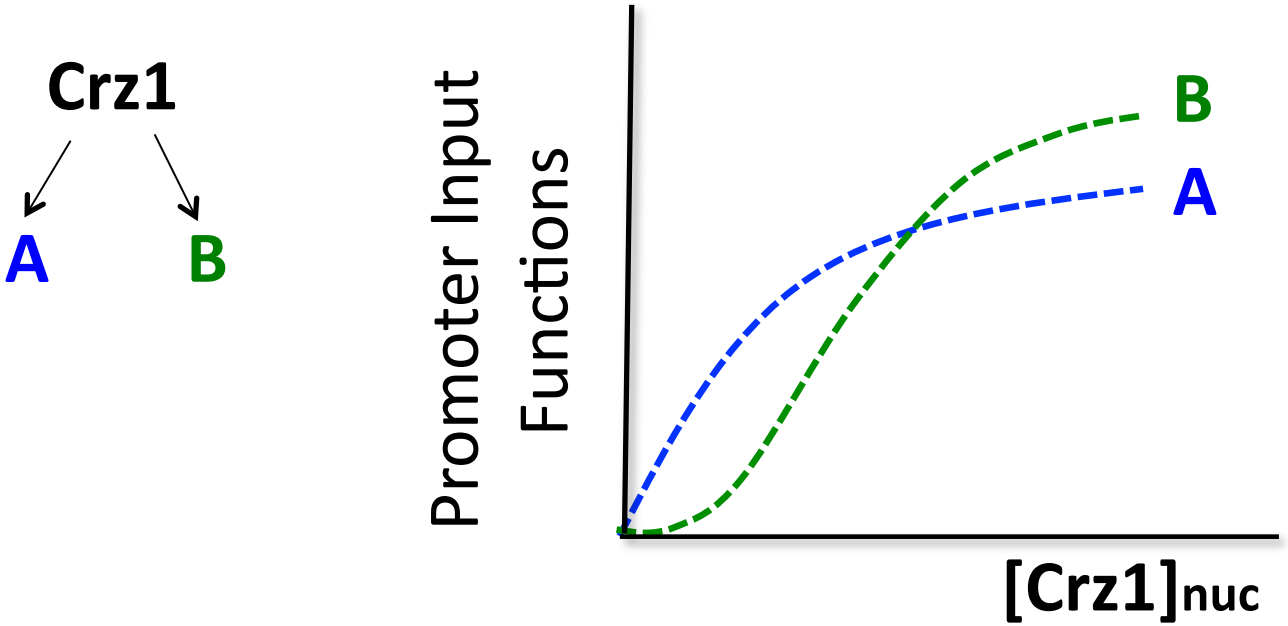
Crz1 encodes signals via Frequency Modulation (FM)



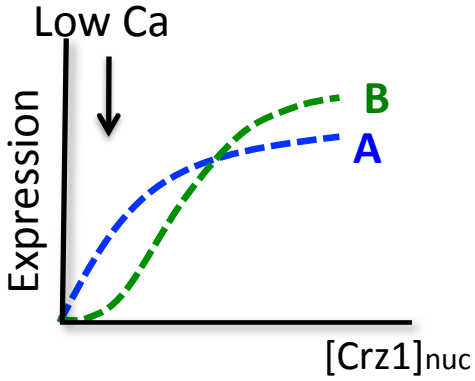
Frequency Modulation (FM)

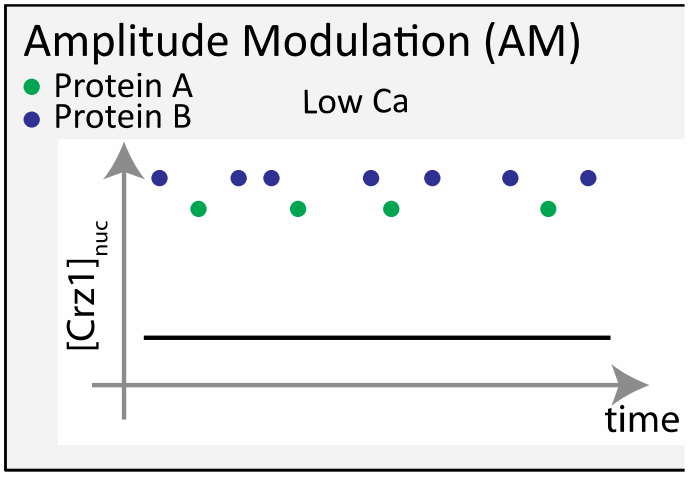
- FM radio: Armstrong, 1933.
  - Less susceptible to noise
- Neurobiology: robust signal propagation
- Rocket thrusters: “Bang bang” control

Crz1 Target Genes Respond Differently to $[Crz1]_{nuc}$



AM Fails to Coordinate Expression





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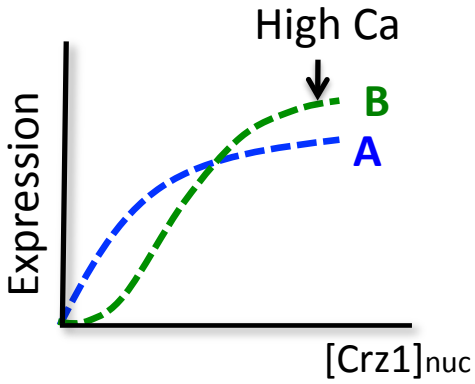
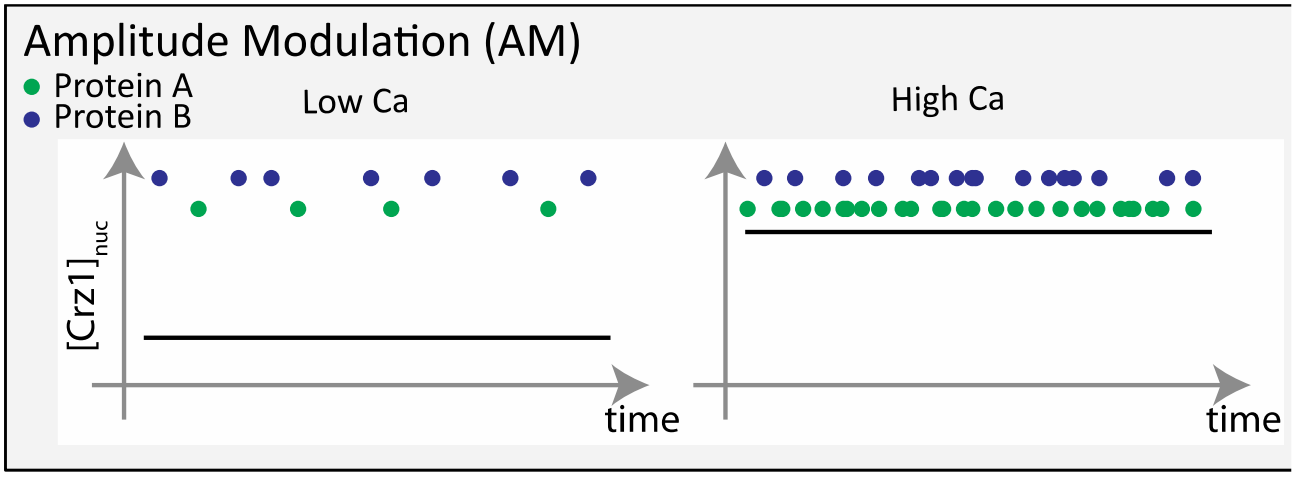
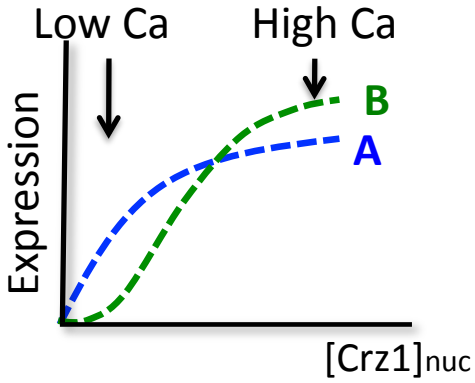
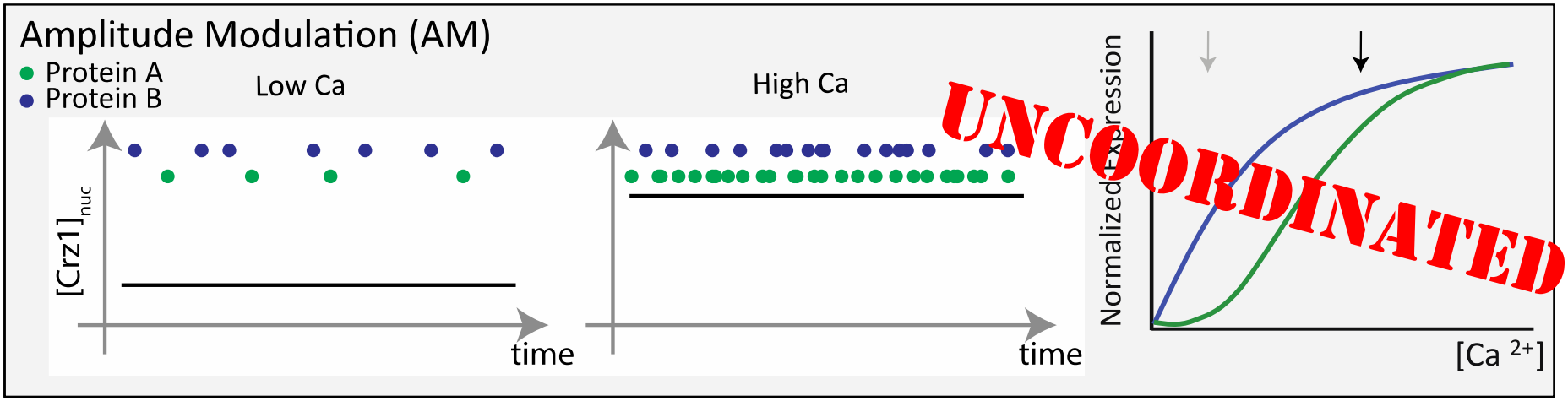


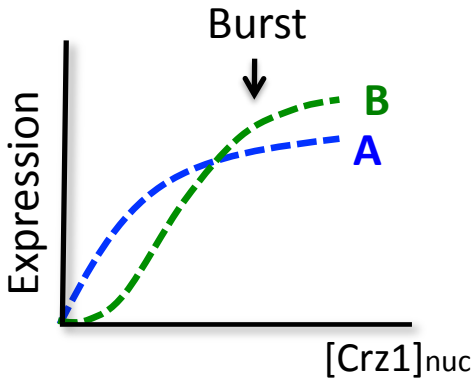


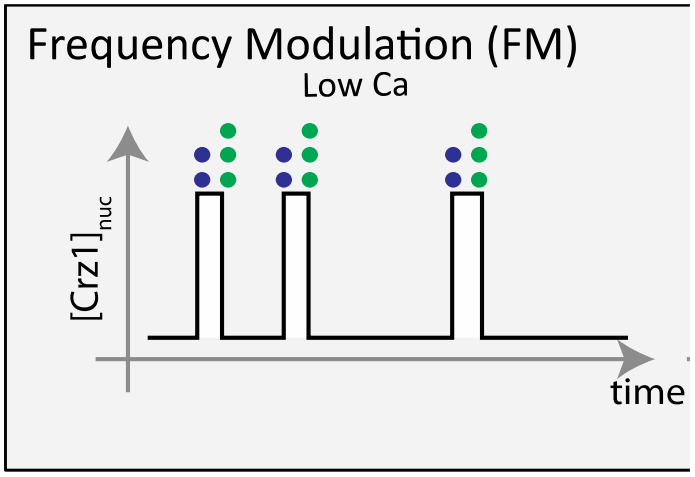
AM Fails to Coordinate Expression



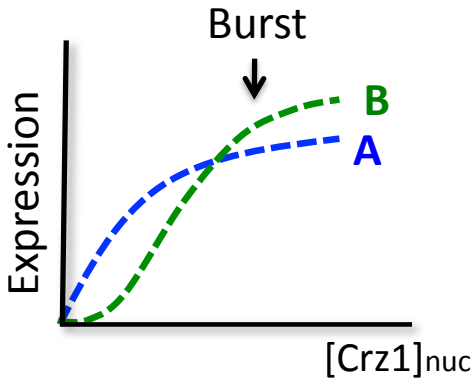


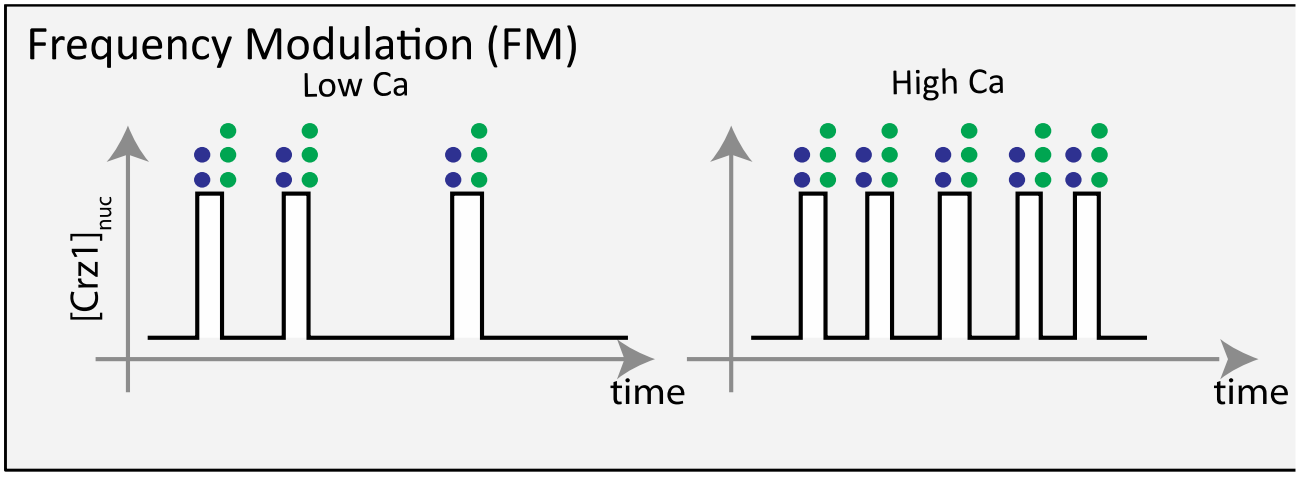
FM Coordinates Expression



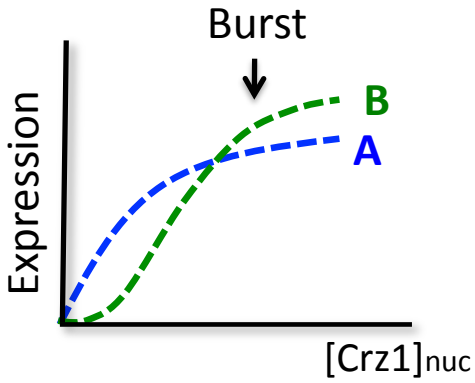


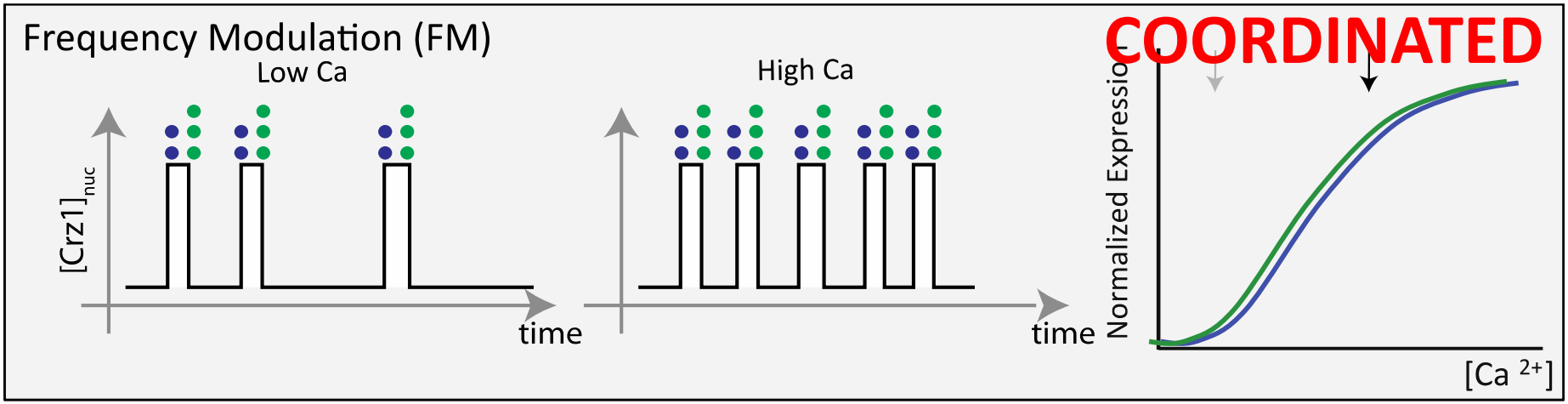
FM Coordinates Expression



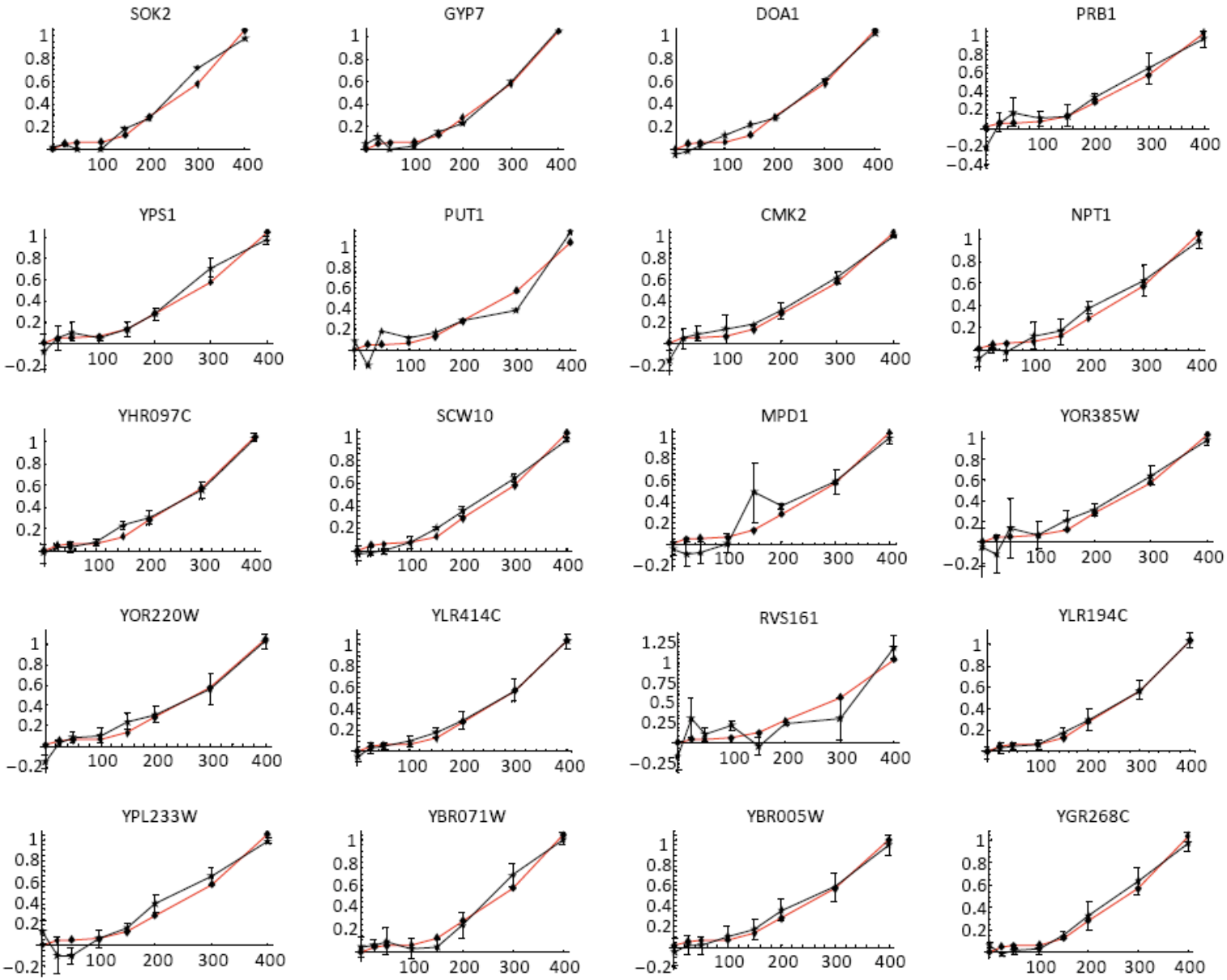


FM Coordinates Expression





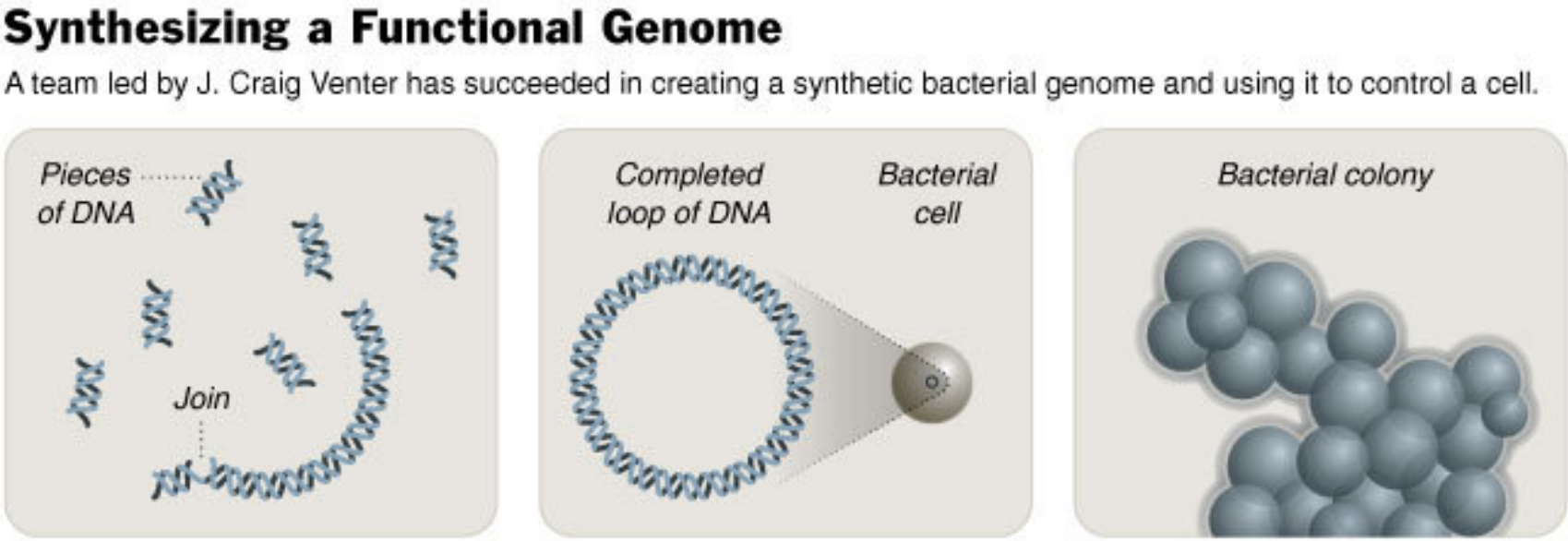
Natural Target Genes Are Coordinately Regulated



Life at the Single-Cell Level…

- …is noisy, dynamic, and complex. But…
- The dynamics of individual cells provides new insights into gene circuit design principles
- Design of synthetic gene circuits enables new cellular behaviors

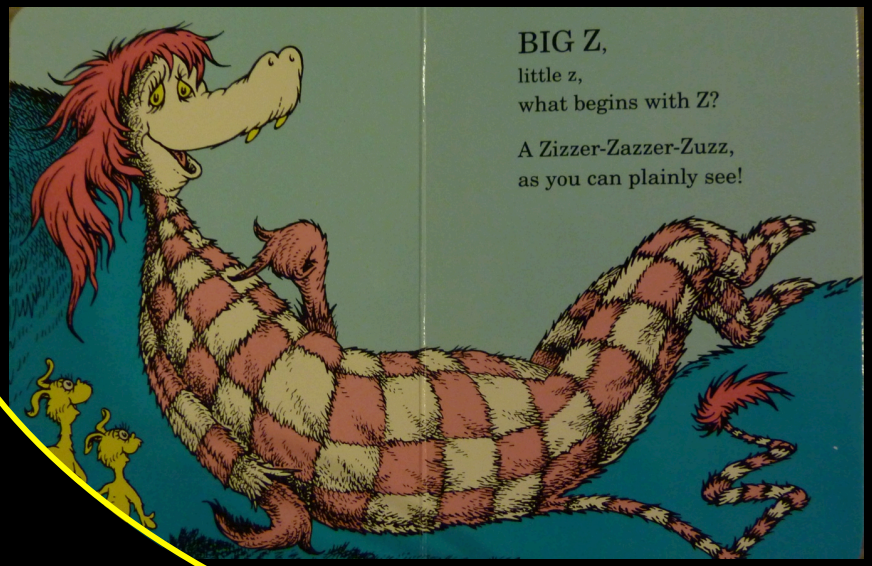
Design and construction of genomes



New York Times

An Expanded View of Biology

Potential Organisms



Natural Organisms

See Elowitz & Lim, Nature 2010

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