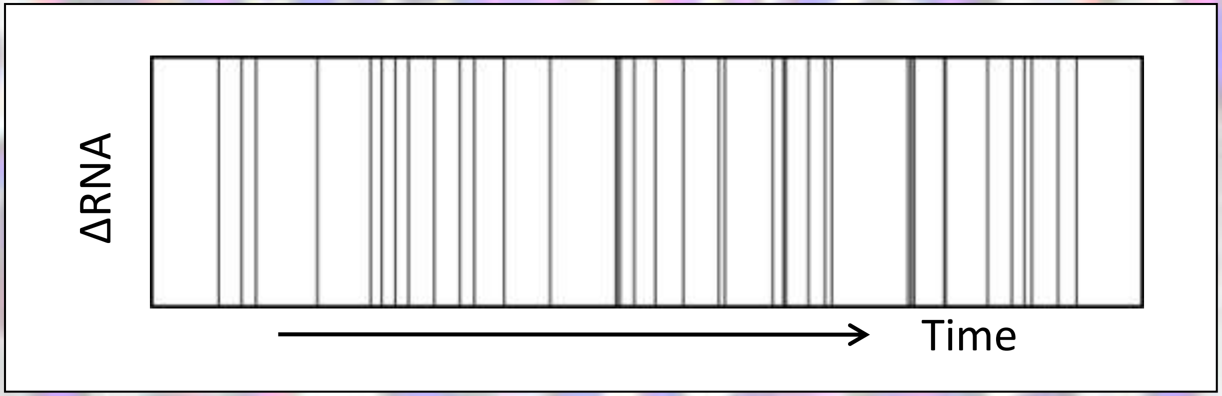
“A physicist studies gene regulation”

Ido Golding
The Verna and Marrs McLean Department of Biochemistry and Molecular Biology
Baylor College of Medicine

KITP 5/2011



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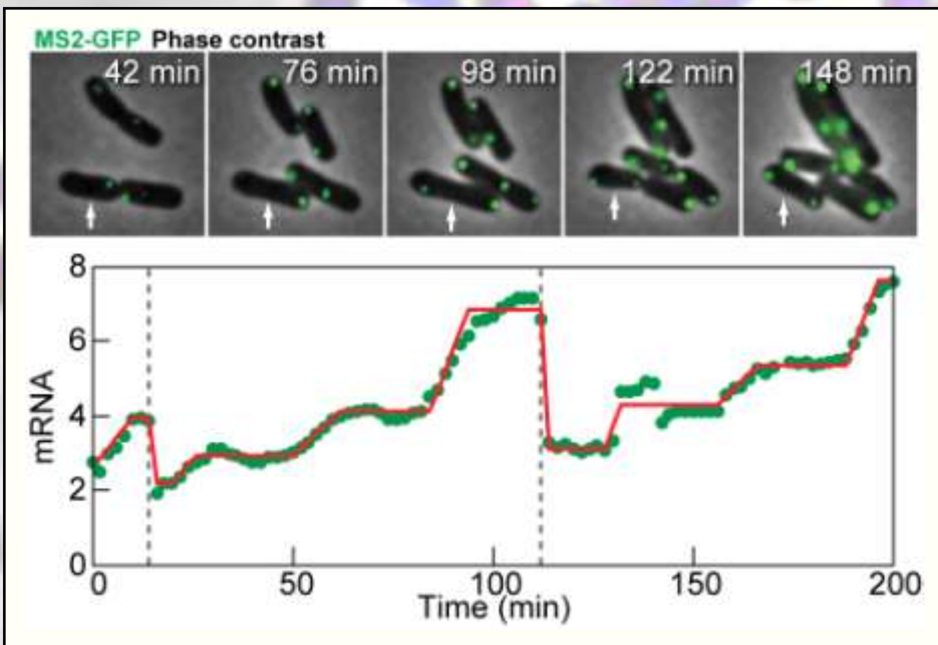
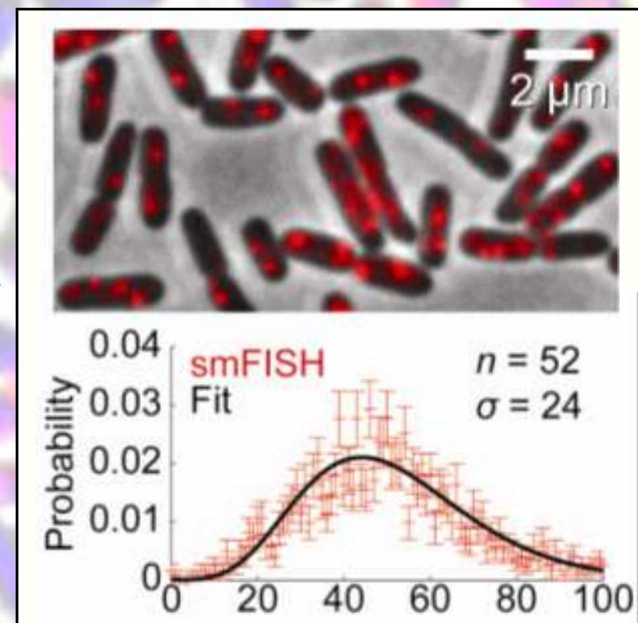
Single-cell kinetics



L.h. So; Figure for Phillips, Kondev & Theriot, *Physical biology of the cell*

Population statistics



L.h. So *et al.*, Nature Genetics (2011)

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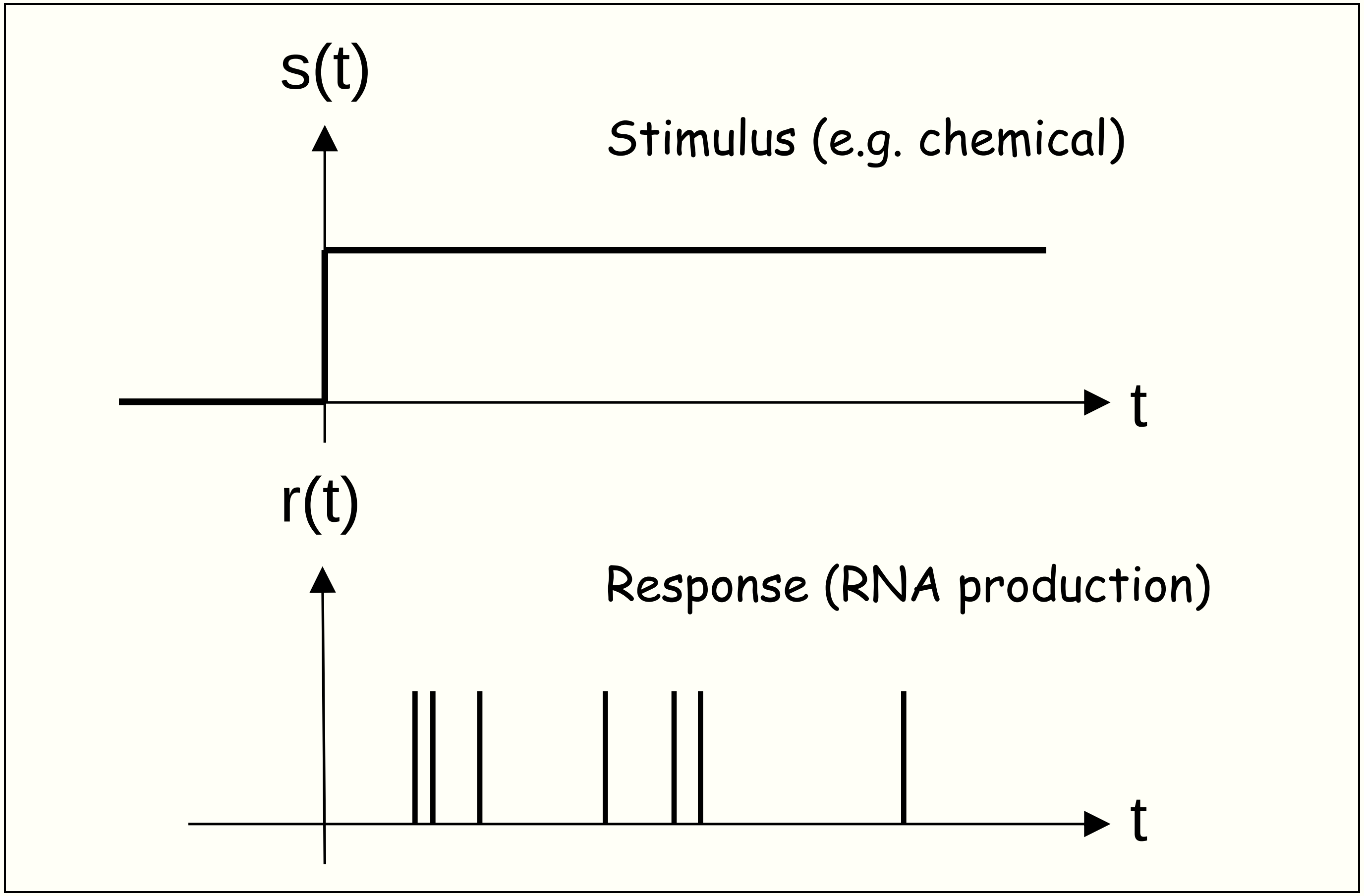
What does it mean for a gene to be 'on'?

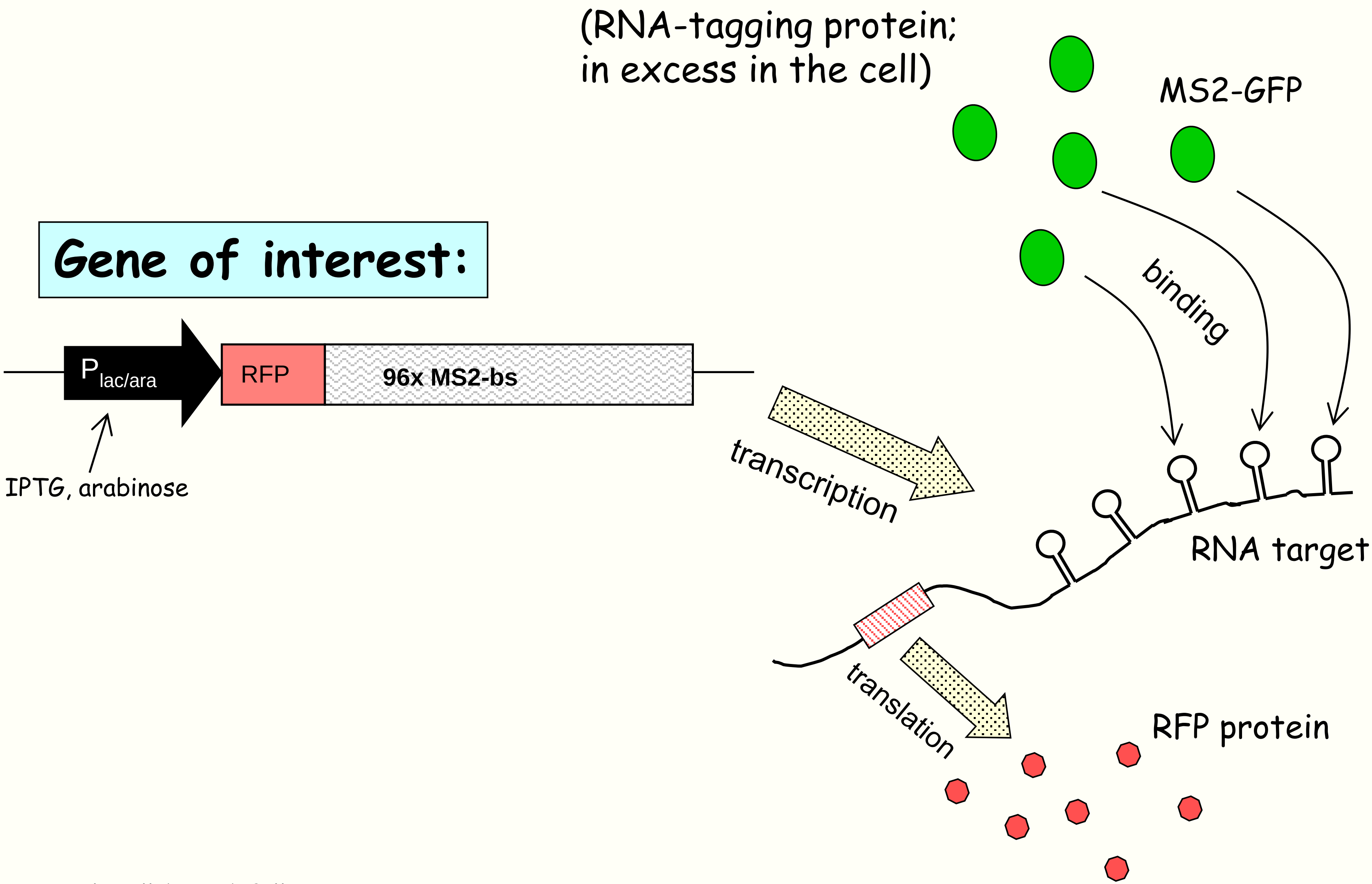


(Subjective choice of scale; coarse-grained)

What are the characteristics of the transcriptional time-series?

Golding and Cox, *Curr. Biol* (2006)

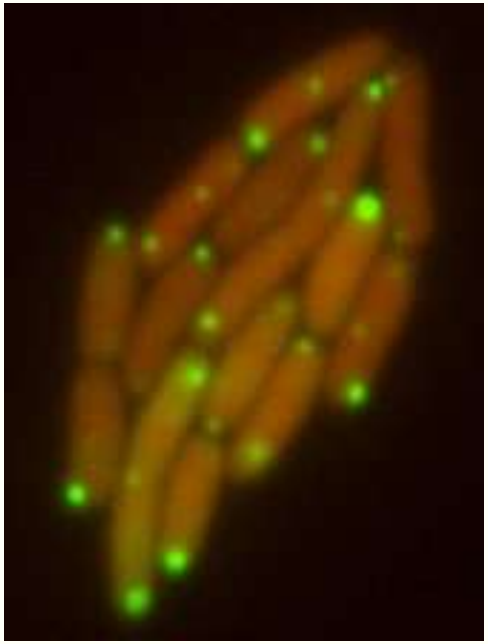
Following transcription in real-time



Golding *et al.*, Cell (2005) following R. Singer

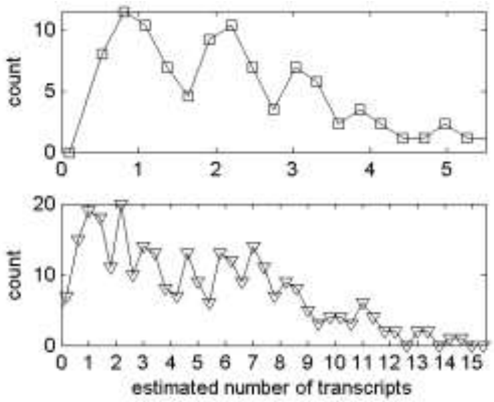
Measuring mRNA & protein numbers

mRNA $\propto$ number of bound MS2-GFPs
$\propto$ photon flux from localized green fluorescence
Protein $\propto$ number of RFPs
$\propto$ photon flux from whole-cell red fluorescence

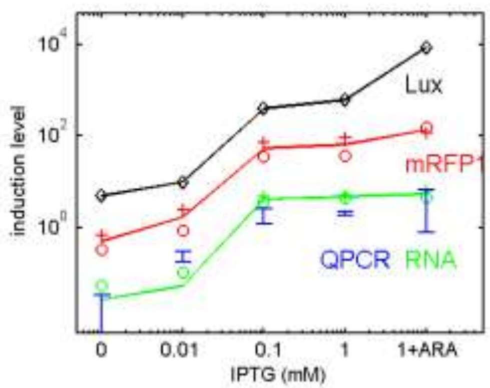


Histogram of
RNA copy number:

1^{st} peak =
inter-peak interval ≈
50-100 X GFP =
1 transcript

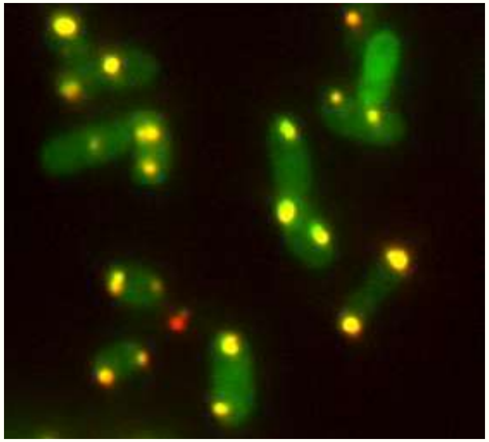


Controls:
QPCR
Protein levels



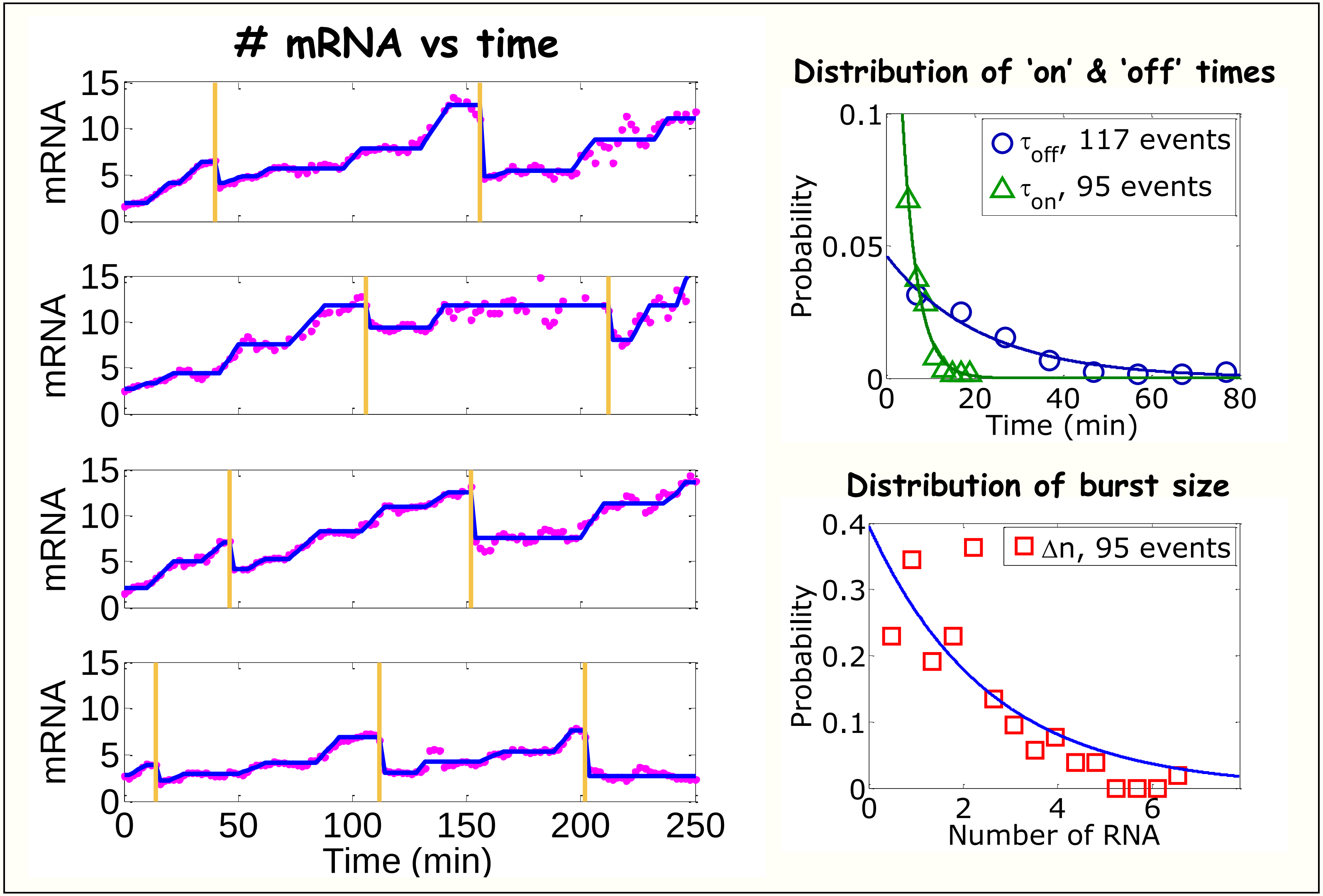
Lux: Lutz & Bujard (1997)

Controls:
FISH



(Thanks to: A. Raj, A. van Oudenaarden)

RNA production occurs in bursts



L.h. So, unpublished; see Golding *et al.*, Cell (2005)

Gene activity can be described as a 2-state process:

$$\mathrm{Off} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{On} \xrightarrow{k_{TX}} \mathrm{mRNA} \xrightarrow{k_d} \Phi$$

Coarse-grained, phenomenological
(Molecular nature of 'on' and 'off' states in unknown…)

Peccoud & Ycart (1995)
Golding and Cox (2006)
Raj *et al.* (2006)
Shahrezaei & Swain (2008)

Bursty gene activity is a universal feature

- Transcription bursts in *E. coli* (Golding *et al.*, Cell 2005)
- Transcription bursts in

  mammalian cells (Raj *et al.*, PLoS Biology 2006)

  yeast (Zenklusen *et al.*, NSMB 2008)

  Dictyostelium (Chubb *et al.*, Curr Biol 2006)

  Drosophila (Pare *et al.*, Curr Biol 2009), …
- Bursty protein production

  (Yu, Xiao, *et al.*, Science 2006; Cai *et al.*, Nature 2006)

Bottom line:

Gene activity is often not Poissonian;
It is pulsatile/bursty/intermittent

Can be described using 2-state model

At this stage only phenomenology…

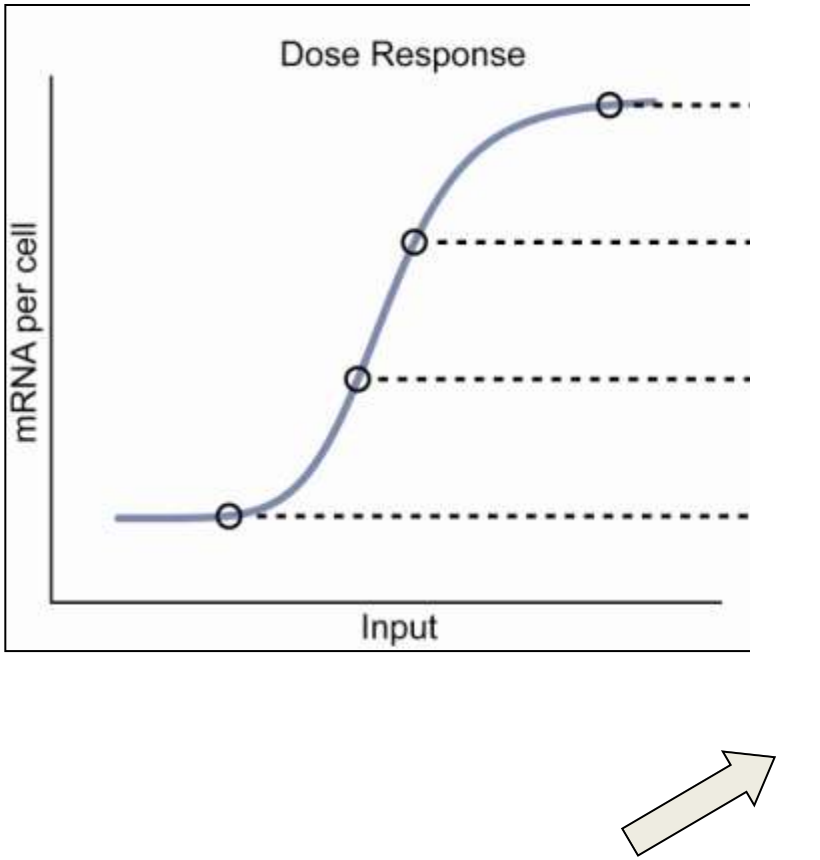
Modulation of the transcriptional time-series

Lok-hang (Tommy) So

Chenghang Zong

Leonardo Sepulveda

Different features of the transcriptional time-series can be modulated to vary gene expression



Time-series modulation is refl

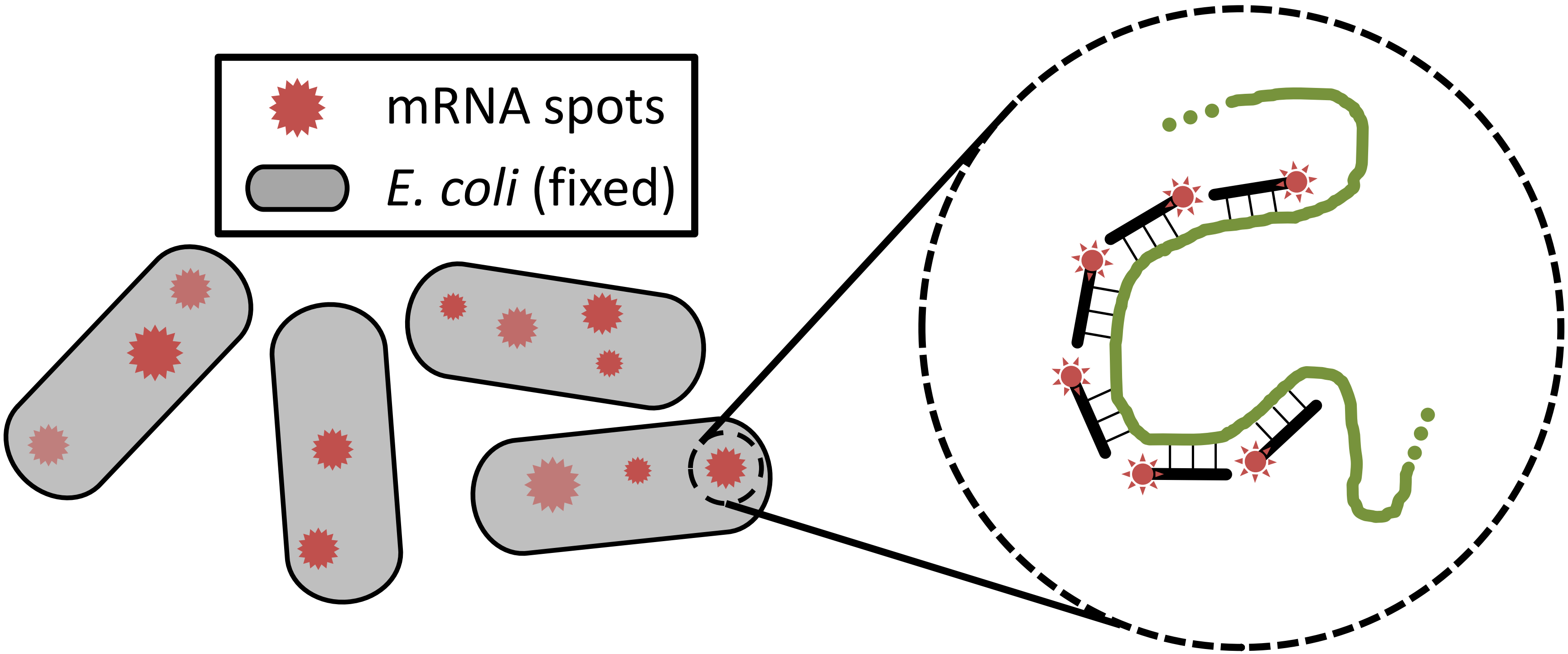
burst size $b = \sigma^2/n$

L.h. So *et al.*, Nature Genetics (2011)

What modulation schemes are found in the cell?

Are different genes modulated differently?

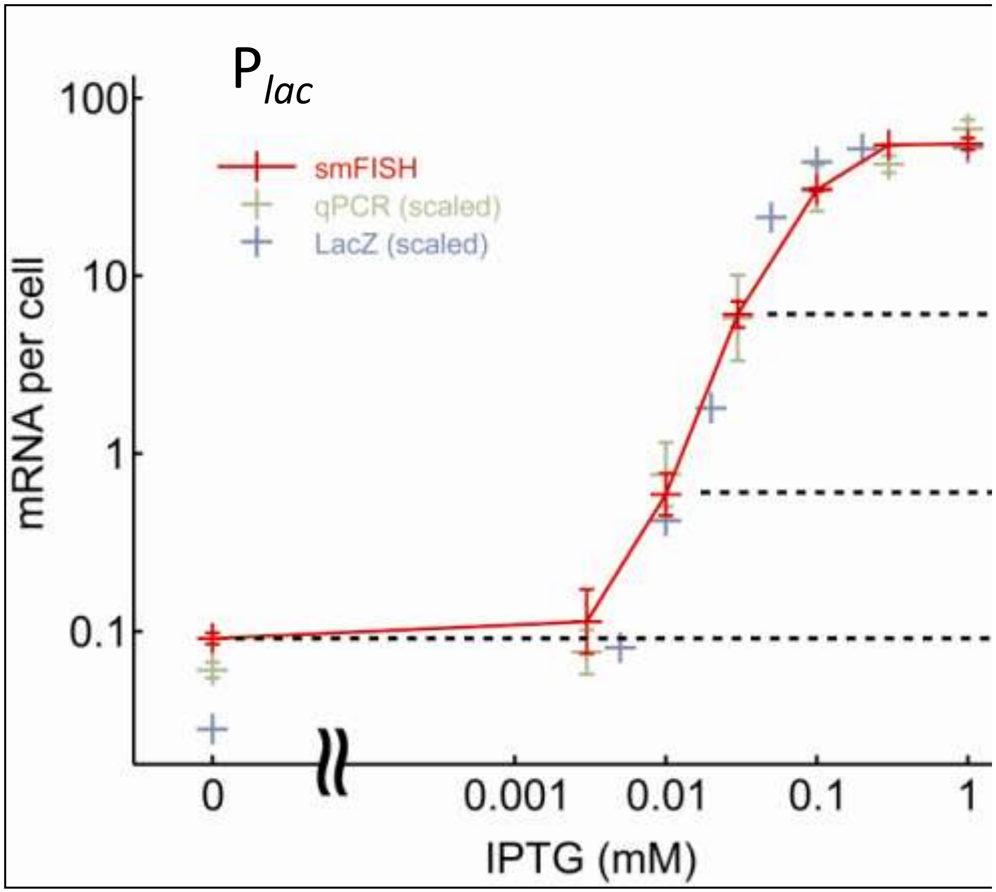
RNA Detection using single-molecule FISH



mRNA copy-number estimated based on total intensity of fluorescent foci

Following Raj *et al.*, Nature Methods (2008)

Characterizing gene activity using smFISH



Well described by
Negative binomial distribution
(2-state model with rapid bursts)

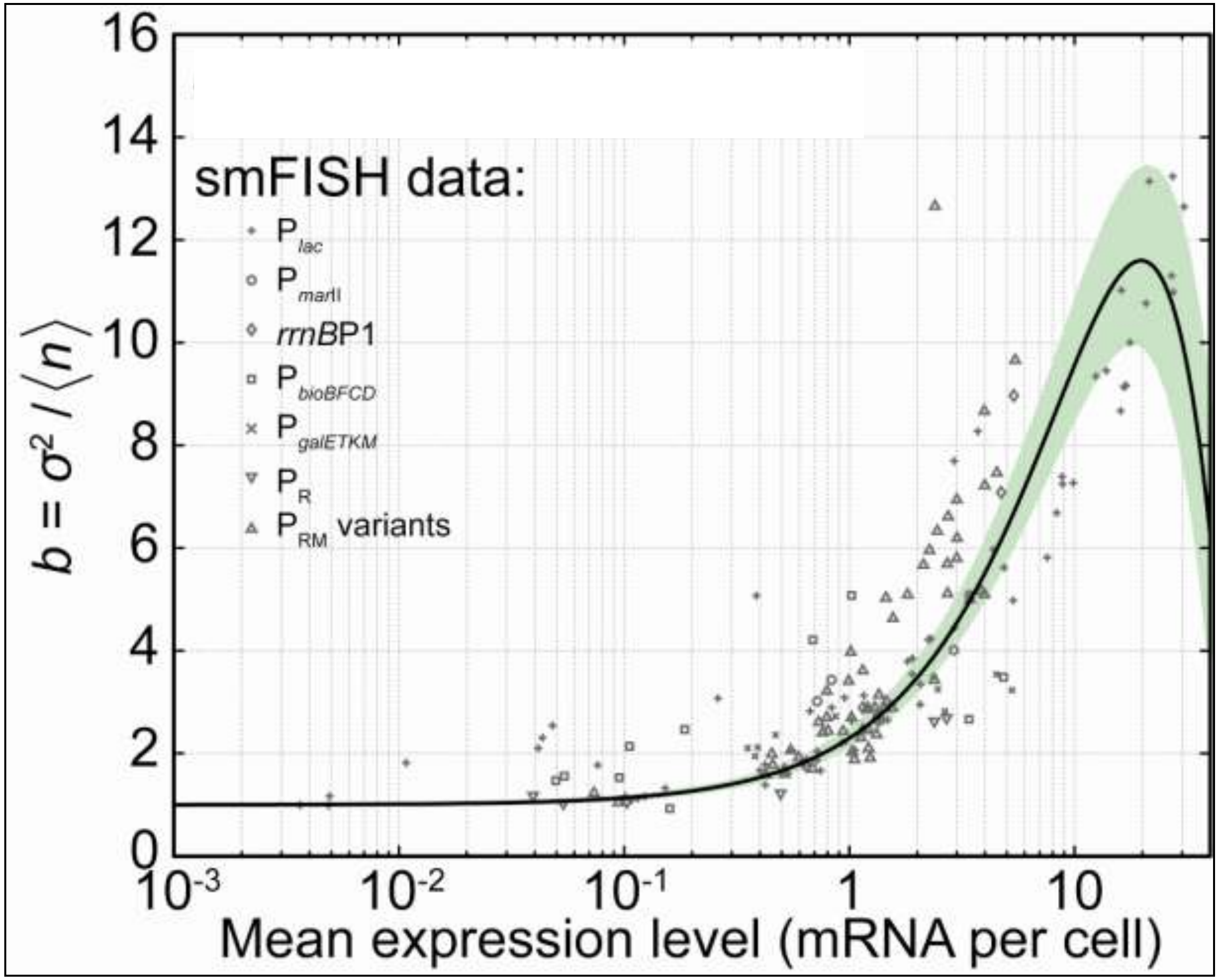
L.h. So *et al.*, Nature Genetics (2011)

LacZ data from Kuhlman *et al.*, PNAS (2007)

Next:
examine different genes…
different expression levels…
different stimuli…
different regulatory mechanisms…

Burstiness exhibits gene-independent behavior

(20 promoters, ~150 experiments)



Data is consistent with modulation of k_{off} alone

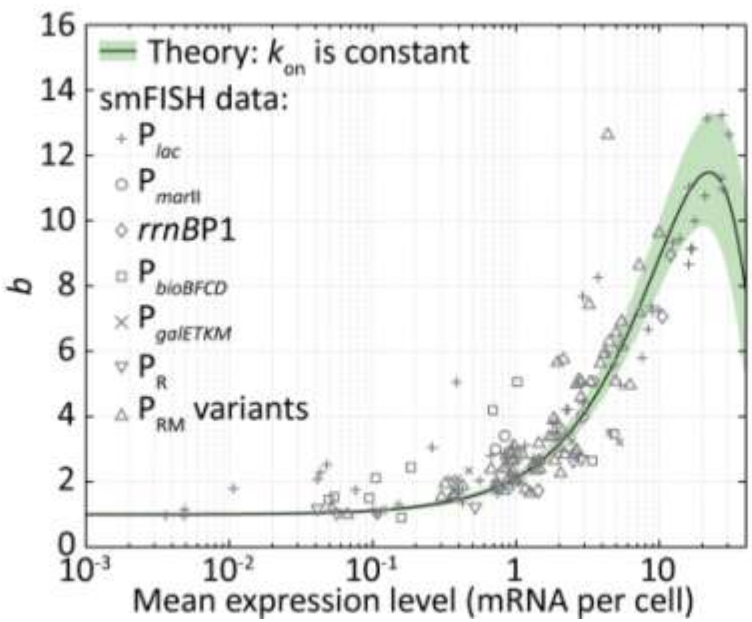
L.h. So *et al.*, Nature Genetics (2011)

Conclusion:

‘on’/‘off’ kinetics do <u>not</u> represent gene-specific features

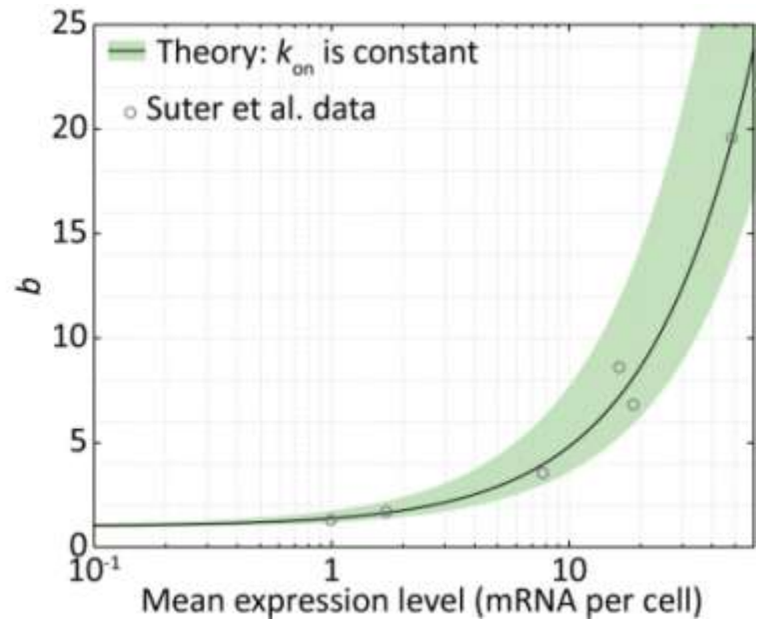
Universality across organisms?

E. coli



So *et al.*, Nature Genetics (2011)

Mouse



Suter *et al.*, Science (2011)

"What is true for *E. coli* is true for an elephant"
—Jacques Monod

Why should we care about the time-series?

Consequences of transactional bursting:

- Information representation by the cell

So *et al.*, Nature Genetics (2011)

- Stability of an “epigenetic” state

Zong *et al.*, Mol. Sys. Bio. (2010)

- Decision-making by individual viruses

Zeng *et al.*, Cell (2010)

Information representation by the cell

Noisy input (e.g. sugar level) → Transcriptional times-series → Noisy output (protein level)

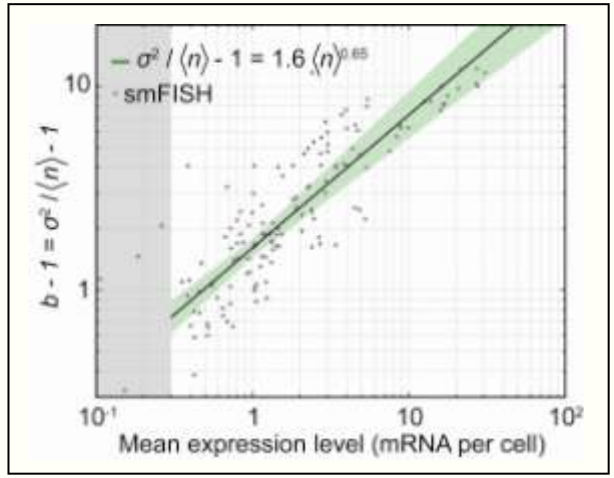
Estimate mutual information between input and output

Ronen Segev & Ananda Ghosh
Collaborators at Ben Gurion University, Israel

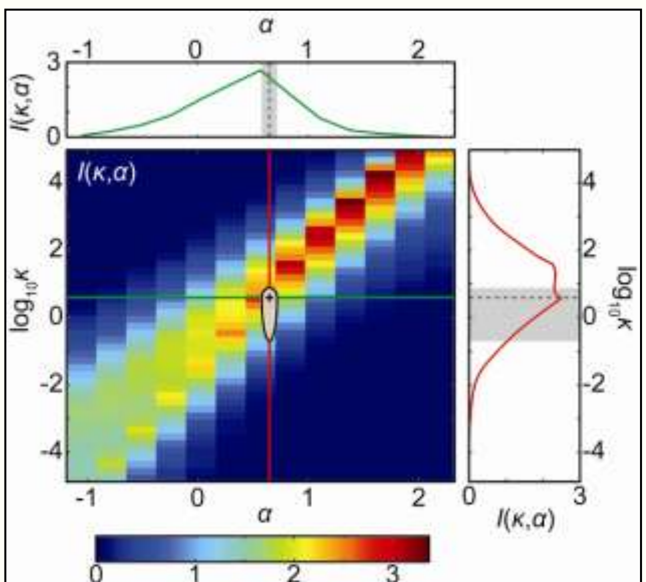
See also: Tkacik *et al.* (2008, 2009)

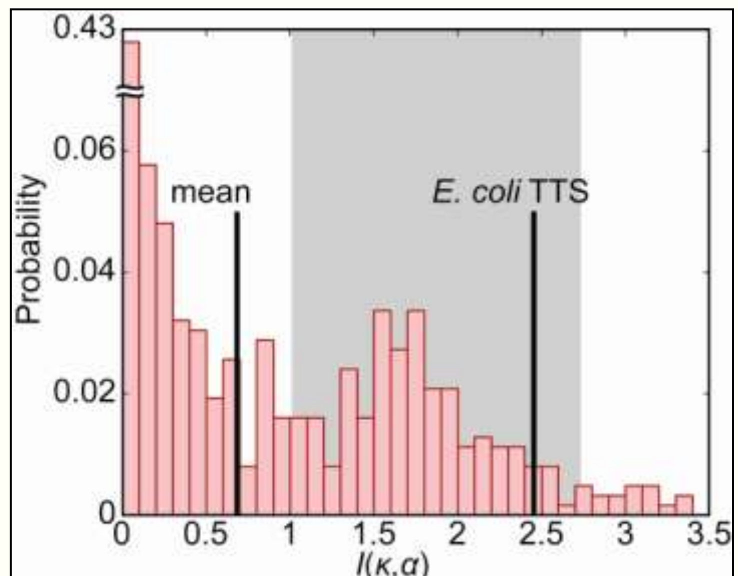
Time-series optimizes mutual information

parameterize time-series
$b(n) \rightarrow \{\kappa, \alpha\}$



Scan "universe" of possible time-series: Actual one is close to optimum





Consequences of transactional bursting:

Stability of an epigenetic state

Chenghang Zong

Lok-hang (Tommy) So

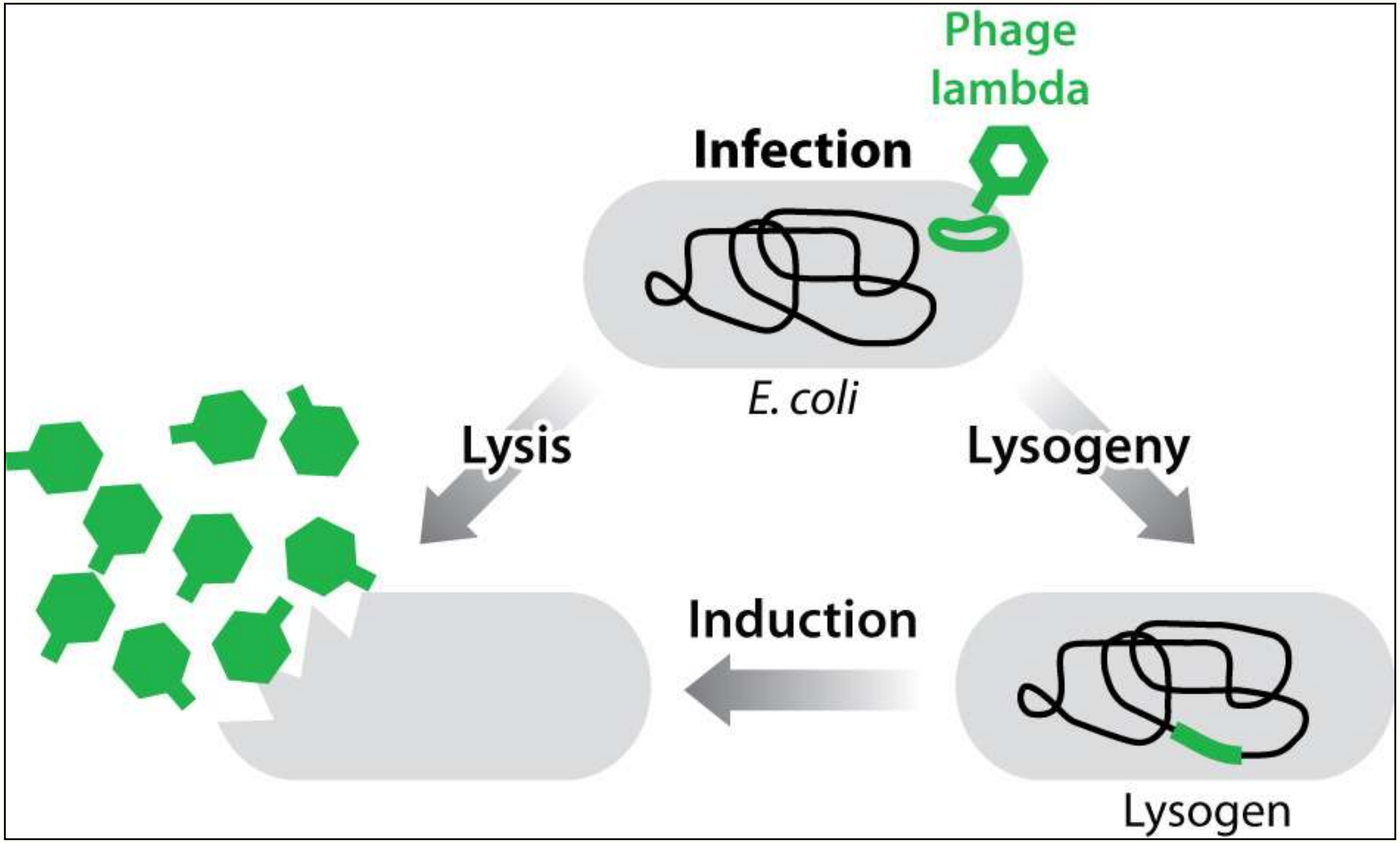
Sam Skinner

Mike Bednarz

Leonardo Sepulveda

Zong *et al.*, Mol. Sys. Bio. (2010)

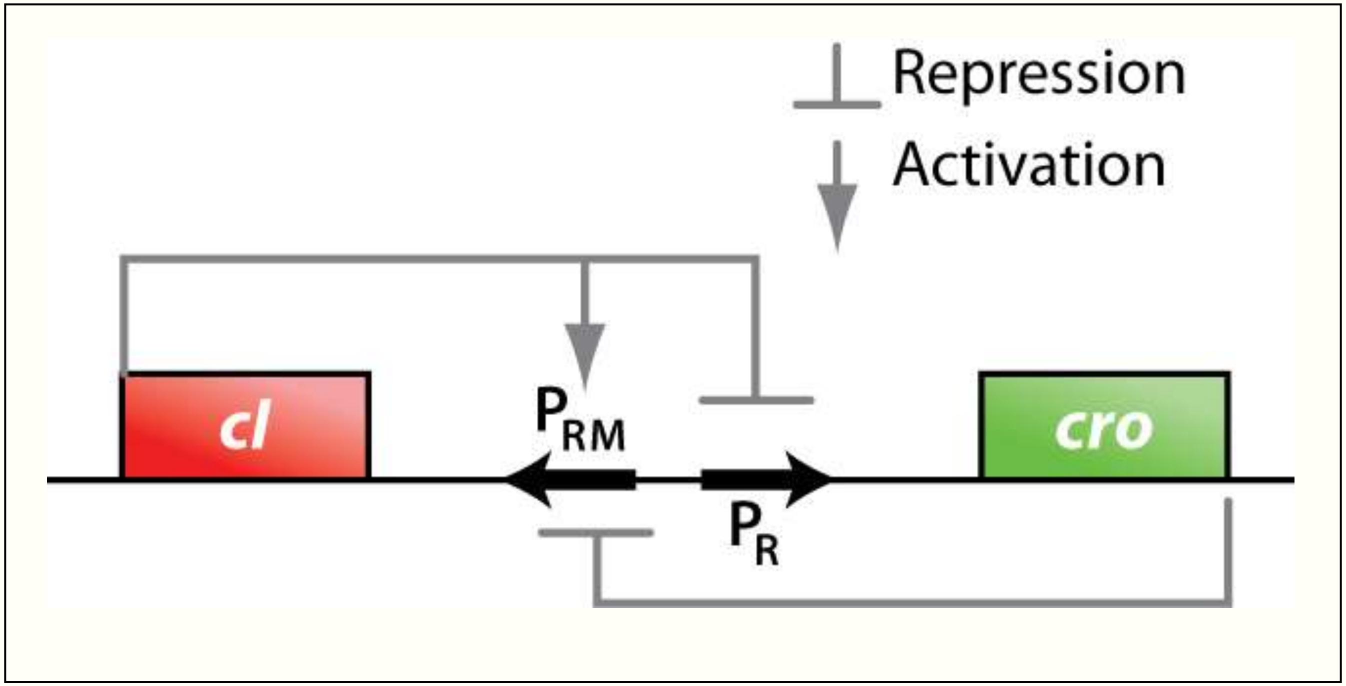
Phage λ: Model system for cell-fate determination



I. Golding, *Annu. Rev. Biophys.* (2011)

Common features with higher systems:
"noisy" decision; epigenetic maintenance by self-regulating TF; high stability / reprogramming

Lysis/lysogeny switch is governed by competition between CI and Cro



I. Golding, *Annu. Rev. Biophys.* (2011)

A very simple model for lysogen stability

- CI is produced in discrete bursts
- Occurrence of bursts follows Poisson statistics
- Cell will switch if no CI is produced during ~1 generation

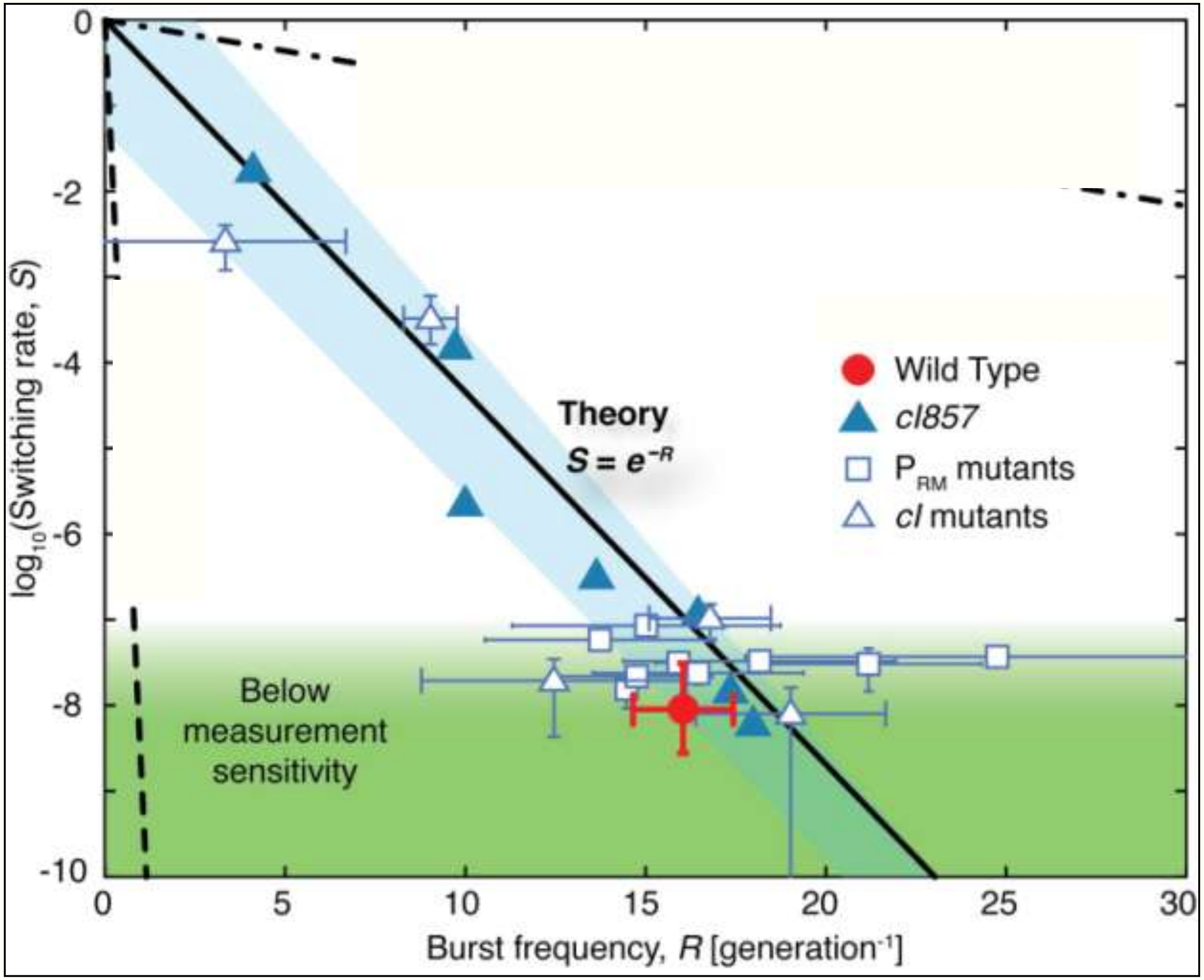
- Therefore: $S = \exp(-R)$

Where S = Switching probability [generation^{-1}]
R = burst frequency from P_{RM} [generation^{-1}]

See also: Bialek (2000); Roma *et al.*, PRE (2005); Mehta *et al.*, Phys. Biol. (2008).

Theory works!

A Simple relationship between <u>switching rate</u> and <u>burst frequency</u>



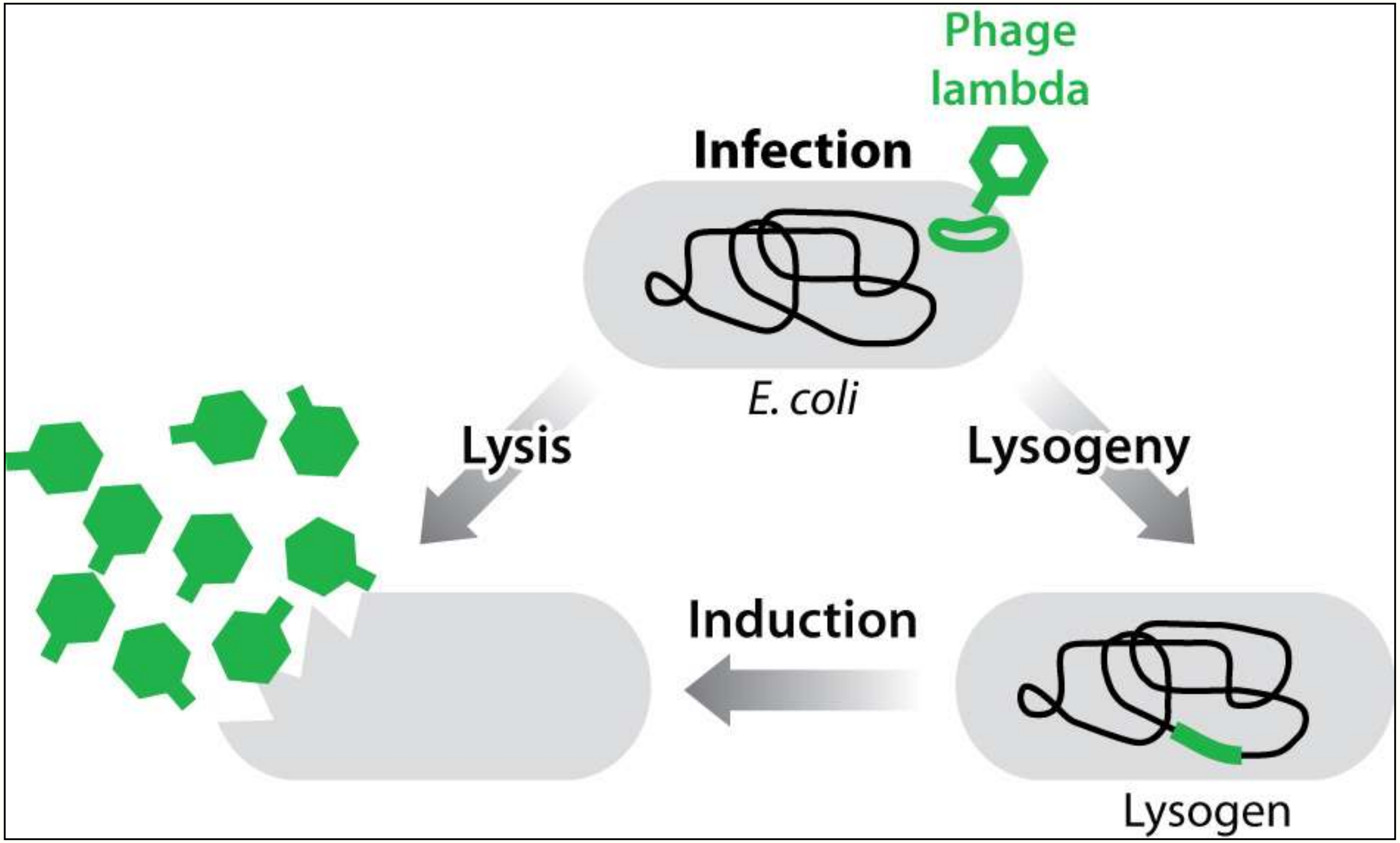
Zong *et al.*, Mol. Sys. Bio. (2010)

(RecA- strain and P_{RM} mutants gift of J. Little)

S : Spontaneous lysis rate

R : from FISH

Post-infection decision



Post-infection decision

What creates cell-fate heterogeneity: Stochasticity or "hidden variables"?

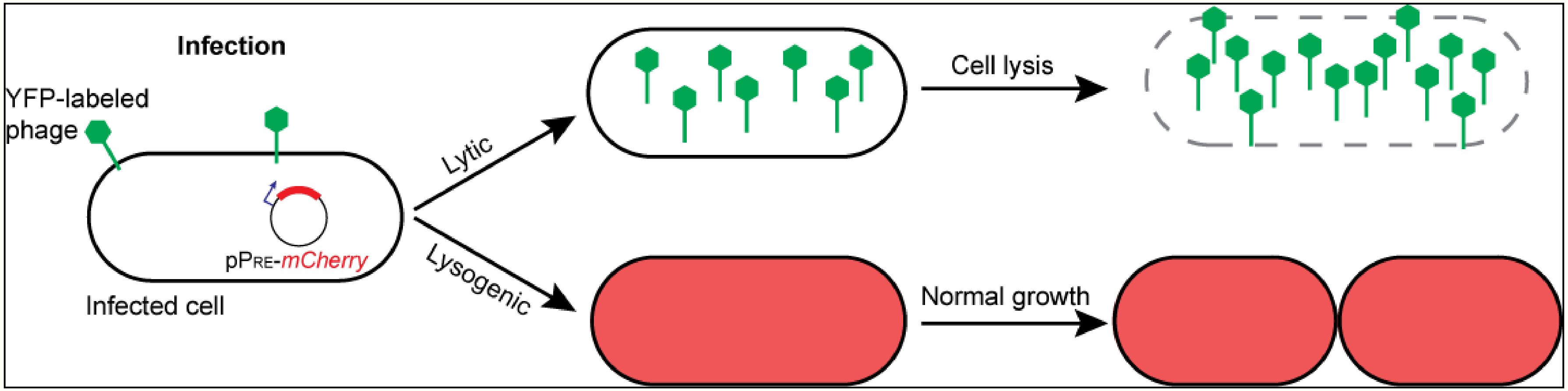
Lanying Zeng

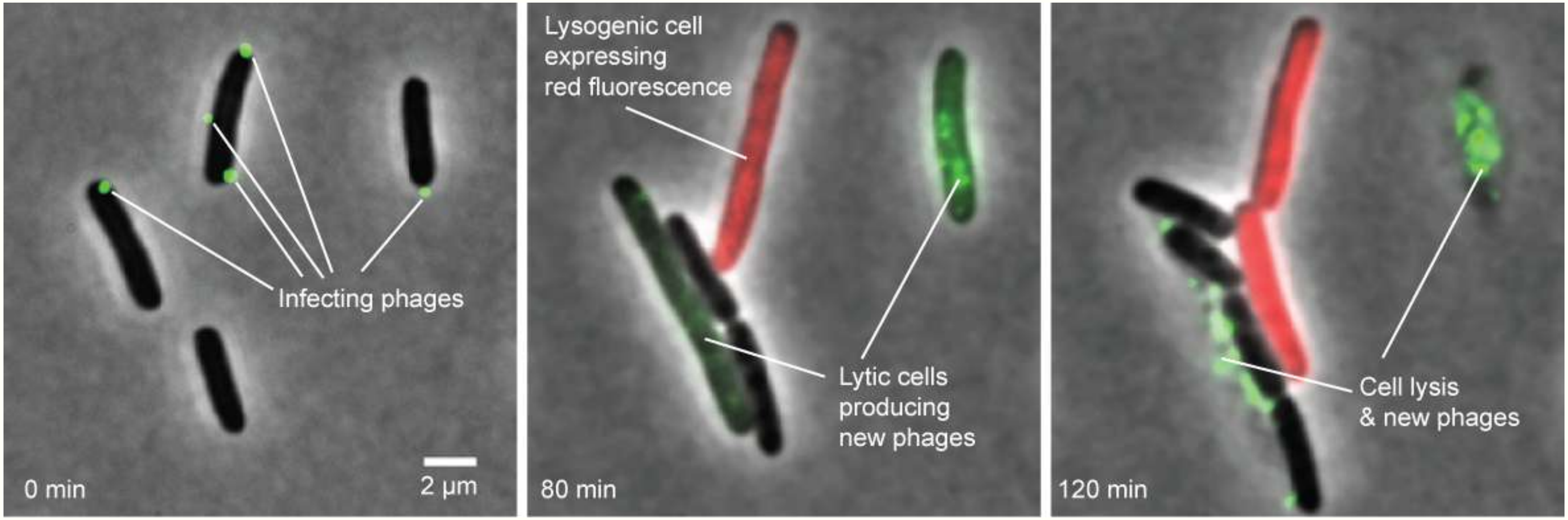
Sam Skinner

+ collaborators:
M. Feiss, J. Sippy
University of Iowa

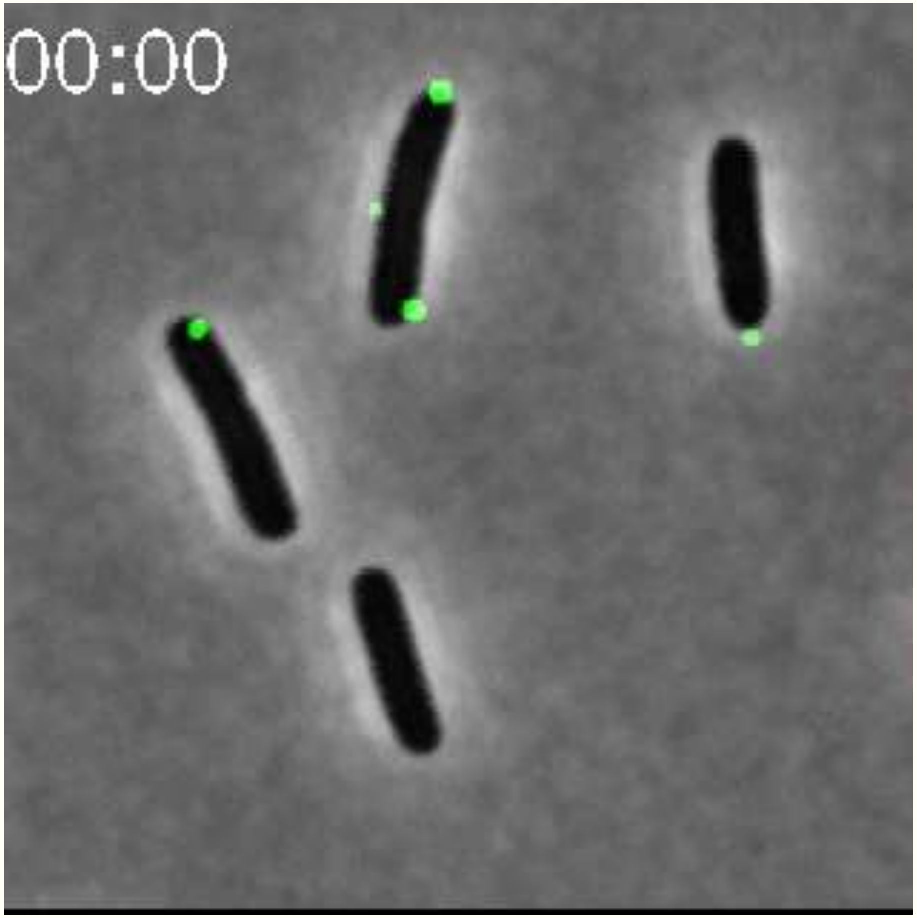
Zeng *et al.*, Cell (2010)

Following cell fate at single-phage/single-cell resolution





Time-lapse movie

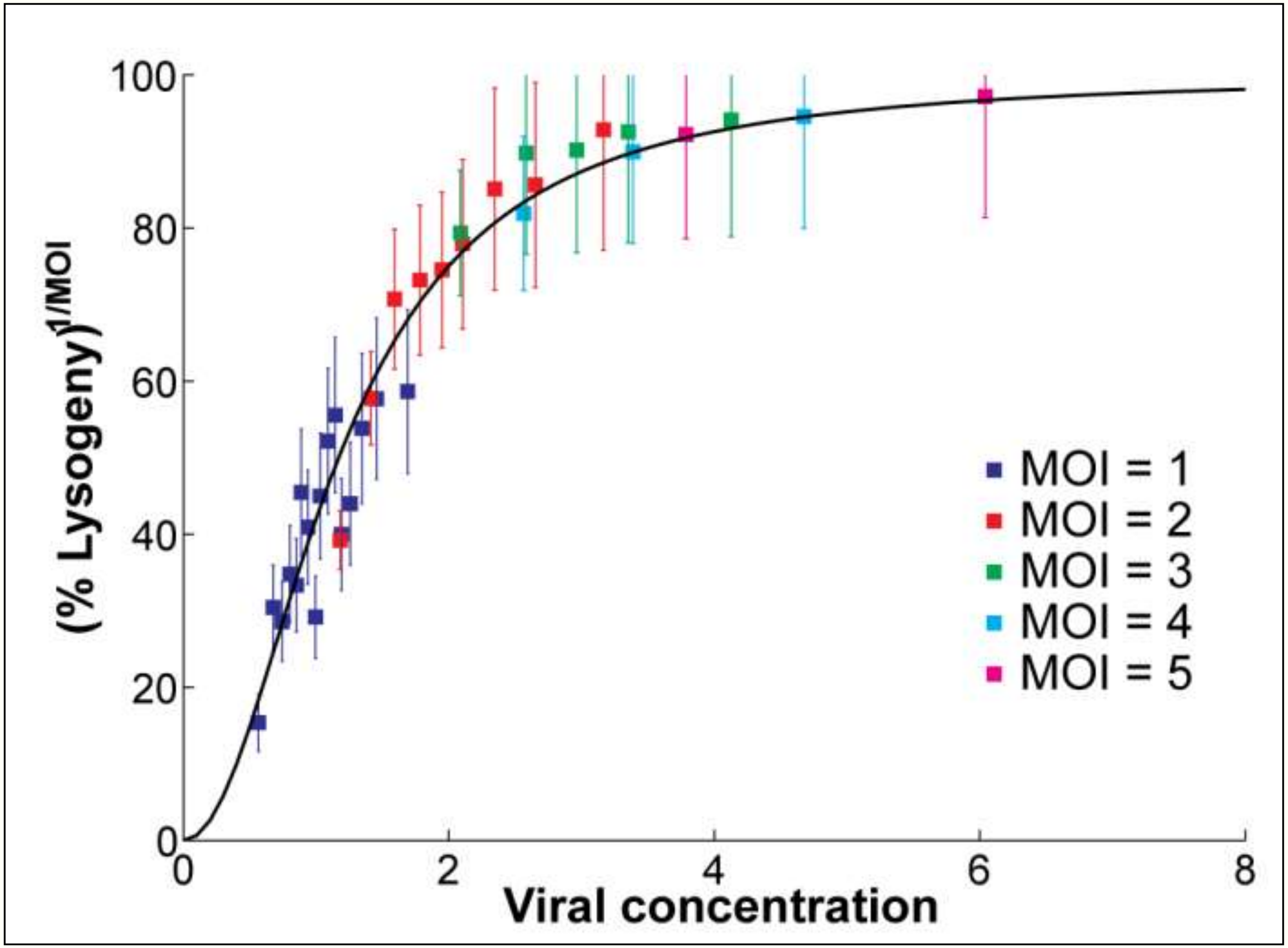


What we found:

* Each phage makes an independent, stochastic decision based on total viral concentration, $f_1(m/l)$
* Only unanimous vote by infecting phages yields cell lysogeny

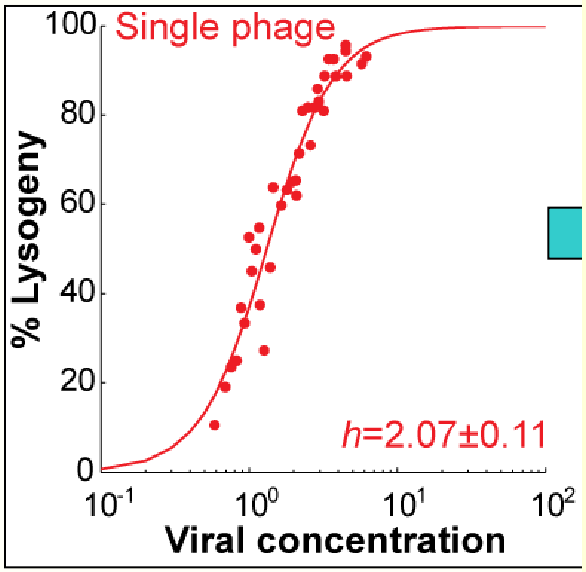
Zeng, Skinner *et al.*, Cell (2010)

Single-phage lysogenization probability $f_1(m/l)$



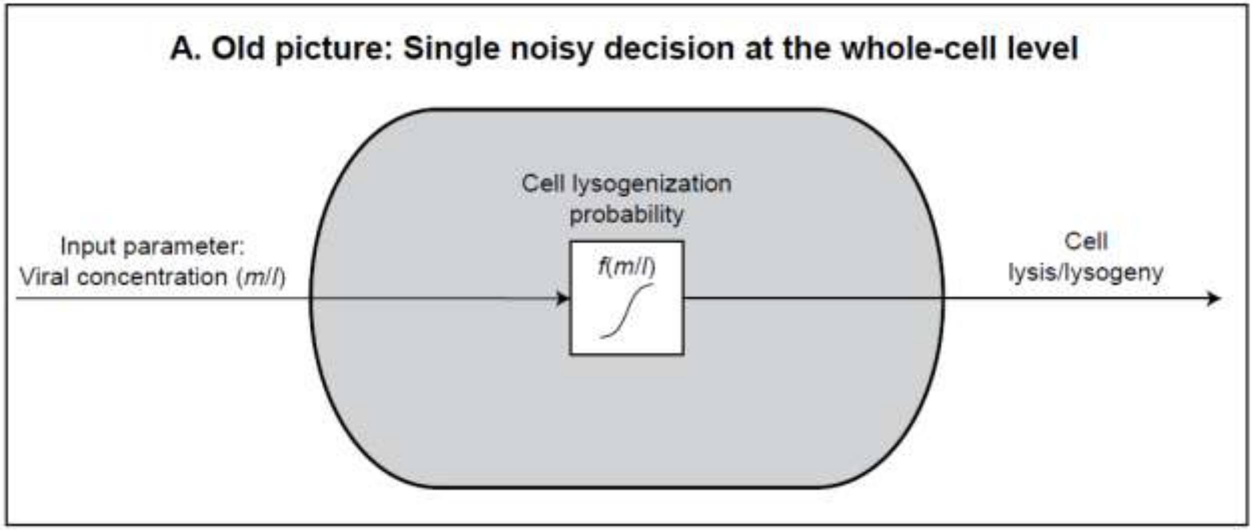
Notice sharpness of decision (Hill≈2)

Theoretical reconstruction of the "coarse-grained" data:



"Noise" arises in transition from single-phage to single-cell,
Not single-cell to bulk.

Decision-making at the sub-cellular level

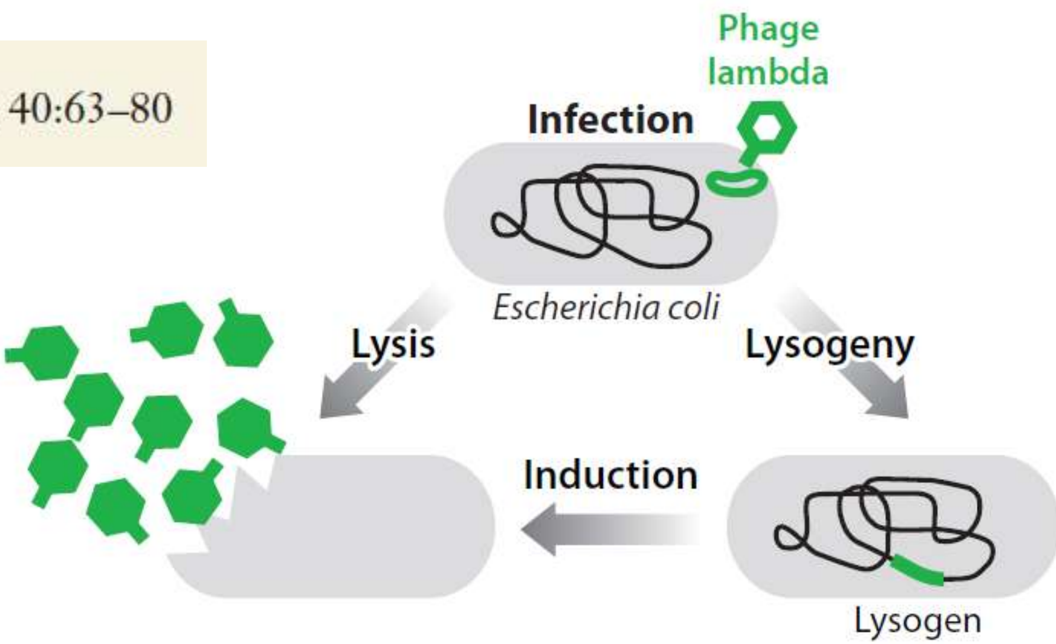


How does decision-making at the single-genome level occur?

Decision Making in Living Cells: Lessons from a Simple System

Ido Golding

Annu. Rev. Biophys. 2011. 40:63–80



RNA FISH

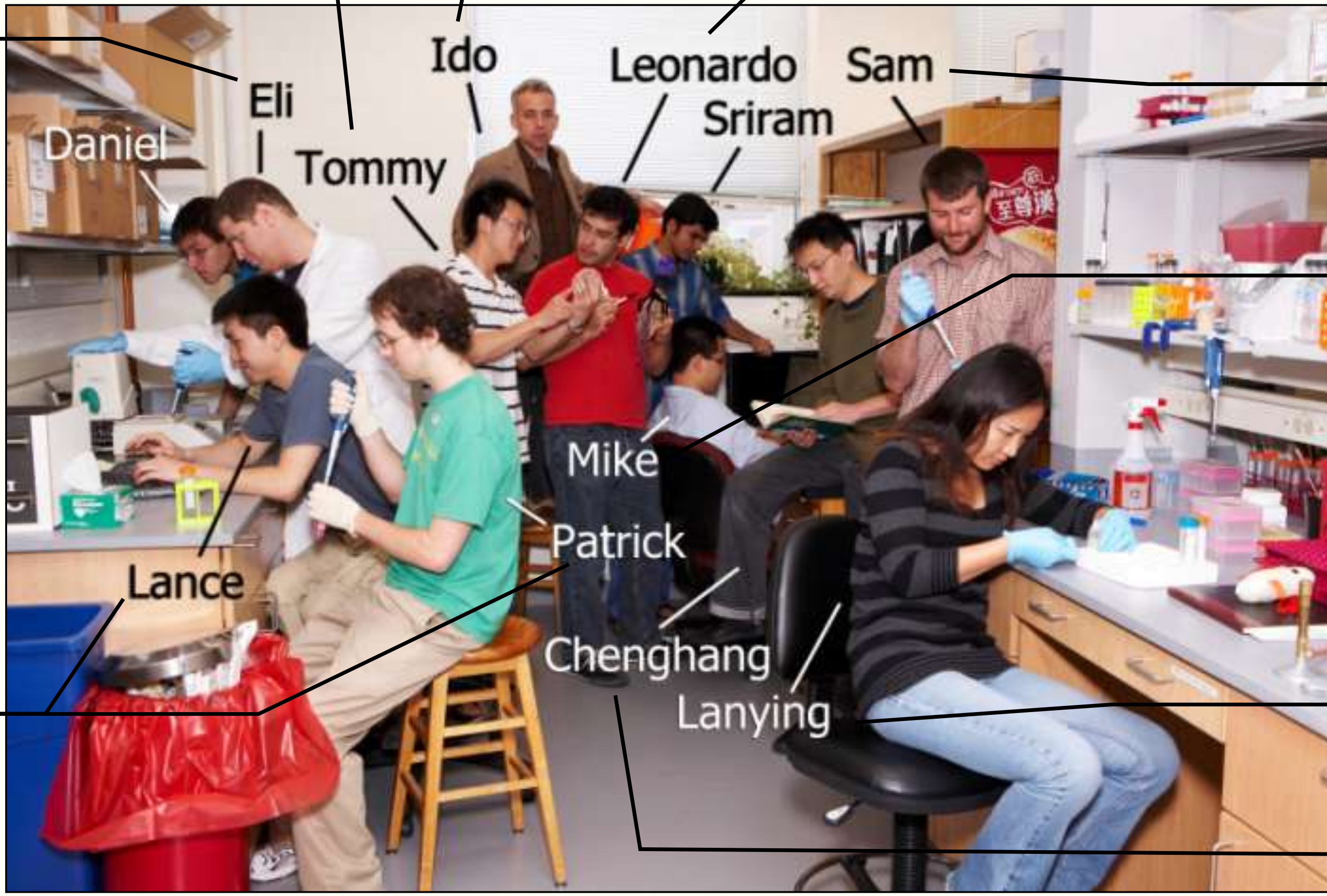
Stressing everyone out

Quantitative immunofluorescence

λ target finding

Post-infection decision

Transcription reporters



Bacterial swimming

Post-infection decision

Lysogen stability

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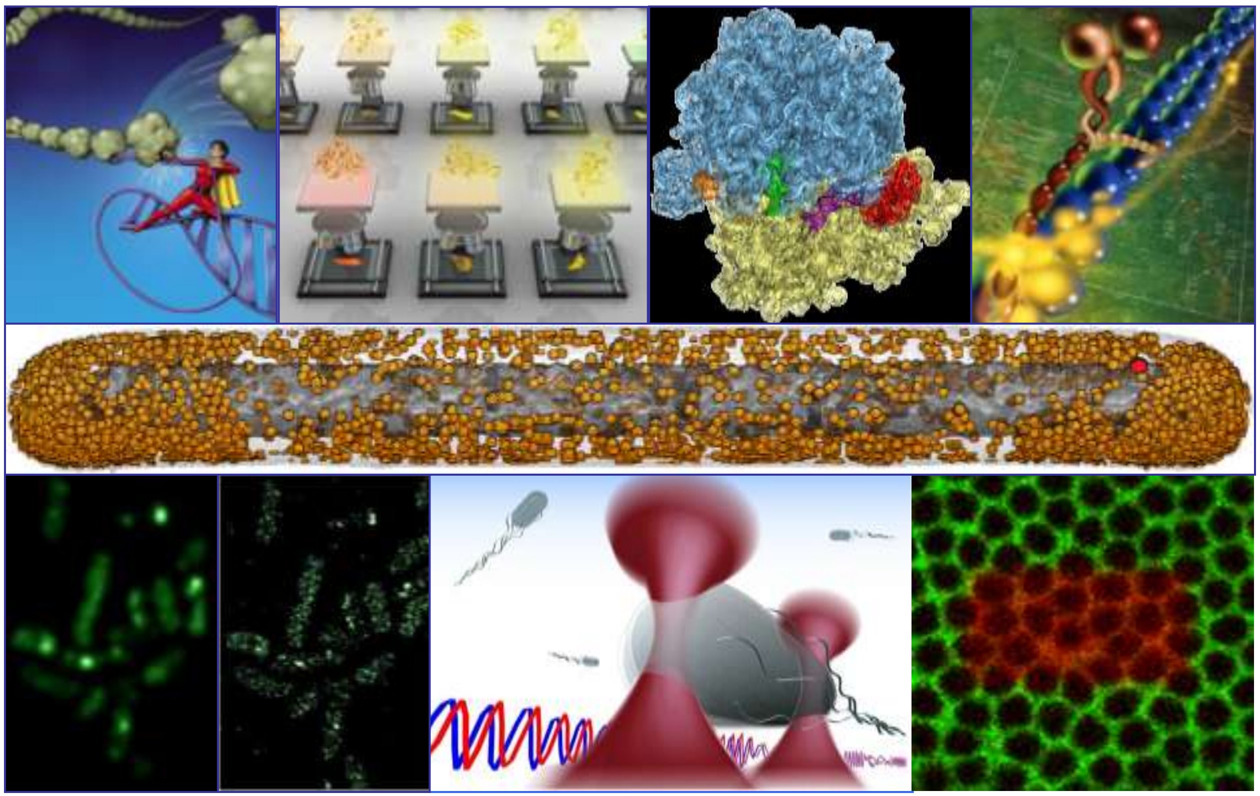

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