# [Non]-deterministic dynamics in cells:
## From multistabilility to stochastic switching

Ádám Halász

Department of Mathematics
West Virginia University

# Cells as machines

- We know a lot about the processes that take place in cells
  - Gene expression (transcription, translation)
  - Sensing, signaling, control of gene expression
- Processes can be described as "reactions"

$$A + B + E \xrightarrow{R(a,b,e,..)} C + D + E$$

  - Molecular species consumed (A,B) produced (C,D), or neither (E)
- Changes are modeled by differential equations

$$\frac{dc}{dt} = -\frac{da}{dt} = R(a,b,e,..)$$

- Issues: uncertainty, parameter variability, stochasticity

# Phenotypes and Steady states

- Genetically identical cells can exhibit different phenotypes
  - Cell differentiation in multicellular organisms
  - Examples in the bacterial world: alternative phenotypes, possibly with a role in survival, adaptation,..
- Due to the different sets of genes that are “on”
- Multiple phenotypes correspond to different equilibria of the dynamical system encoded in the DNA.
- Is phenotype multiplicity always the same as multistability?
- Model predictions may change when including stochastic and spatial effects

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Network of 5 substances

Example of positive feedback in a genetic network discovered in the 50's

This model due to Yildirim and Mackey, based on MM and Hill reaction rates; time delays omitted

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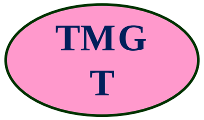

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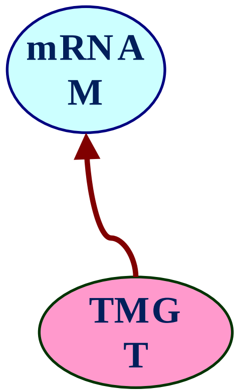

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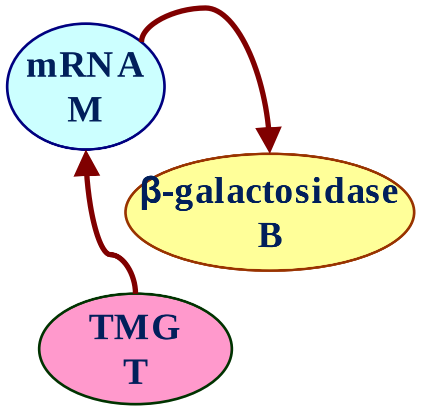

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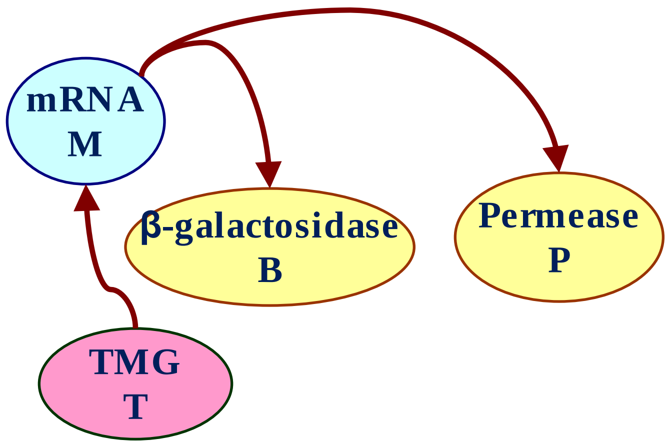

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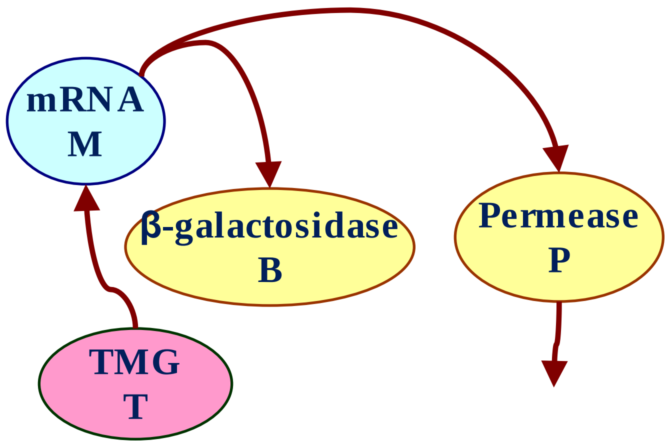

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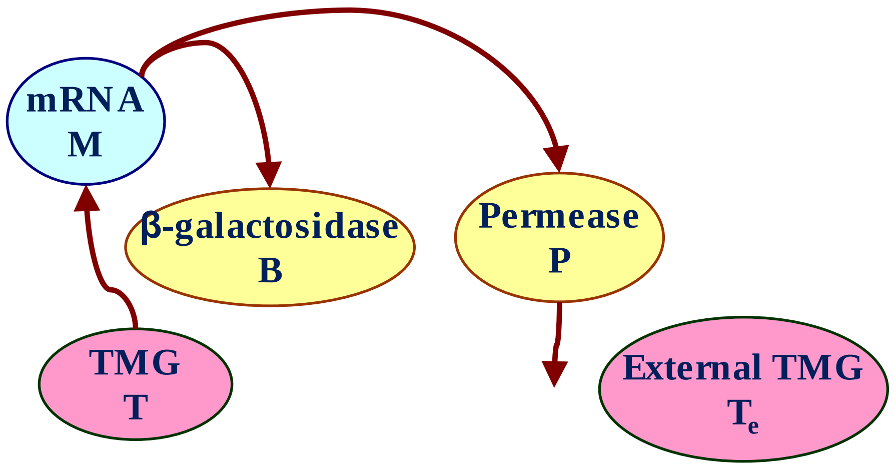

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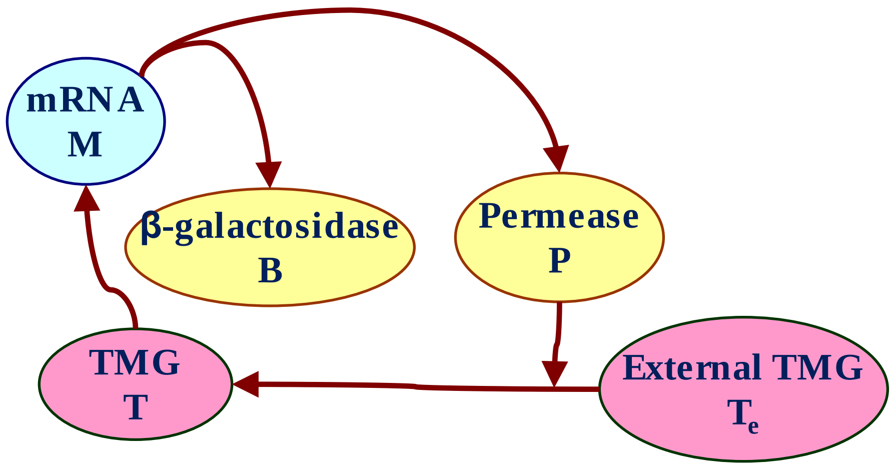

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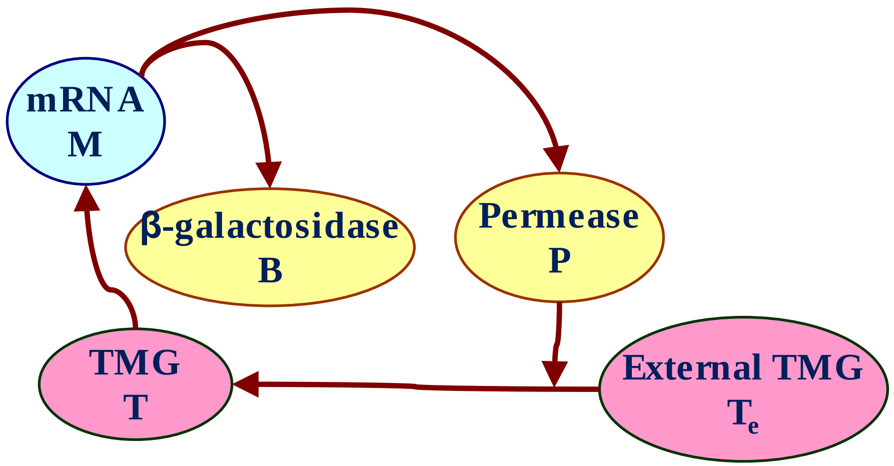


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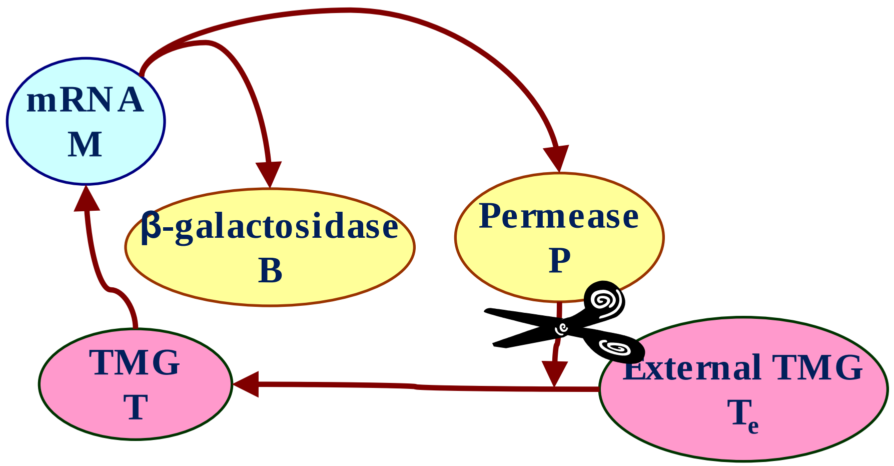


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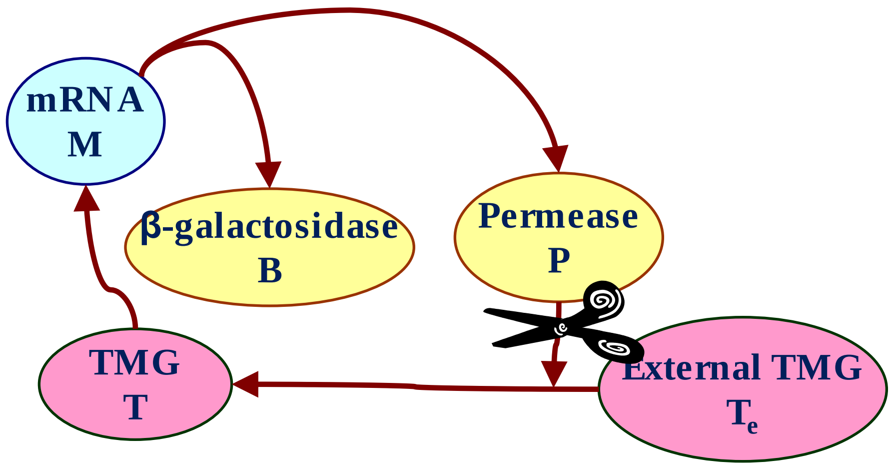


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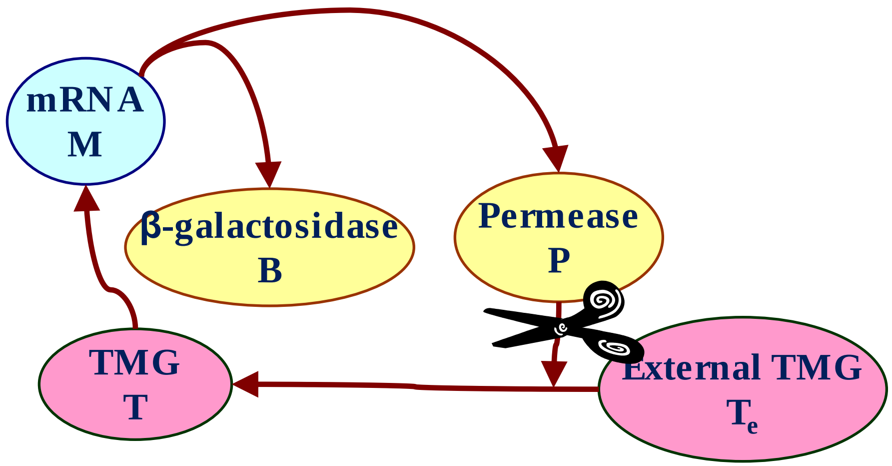


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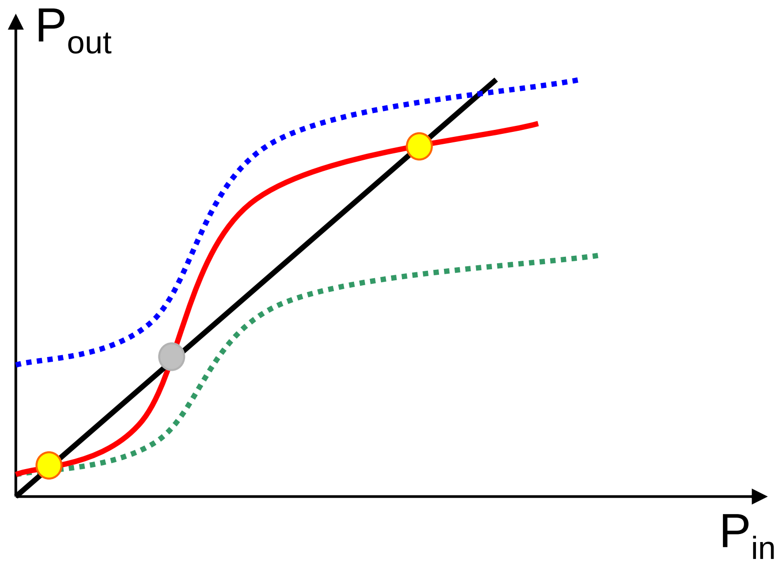

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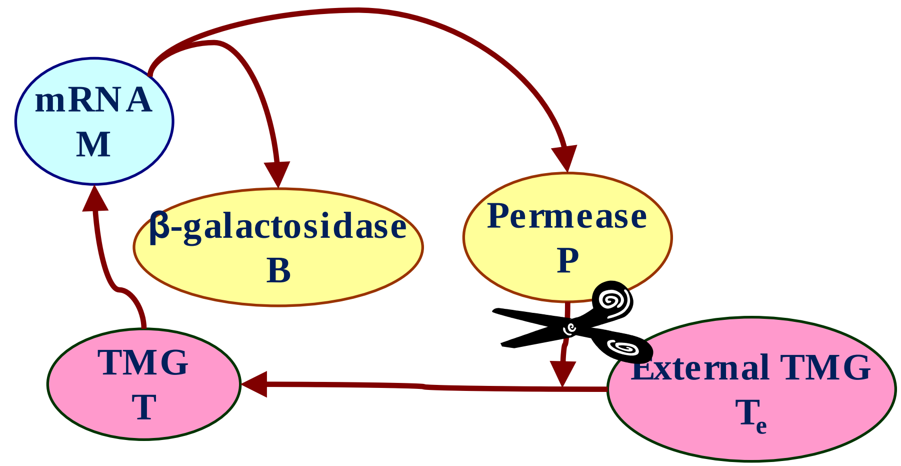


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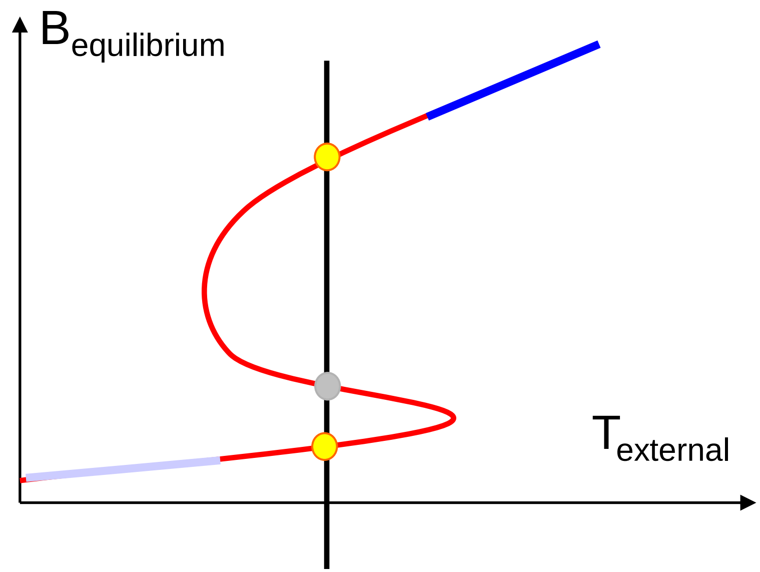

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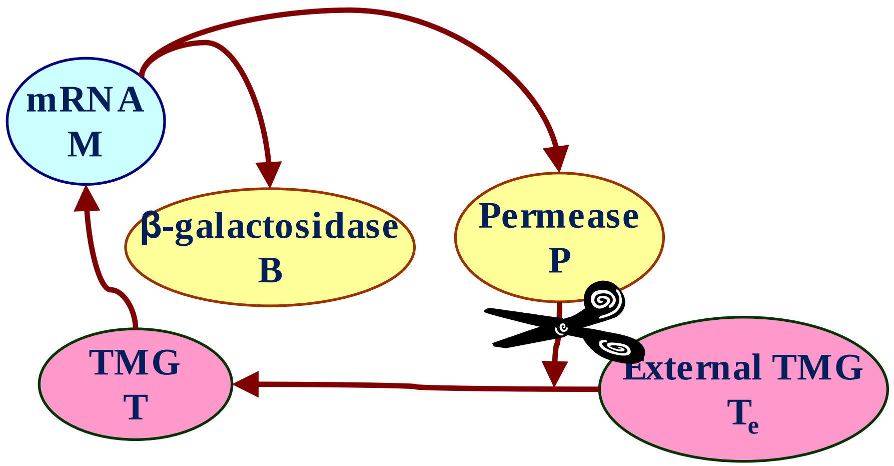


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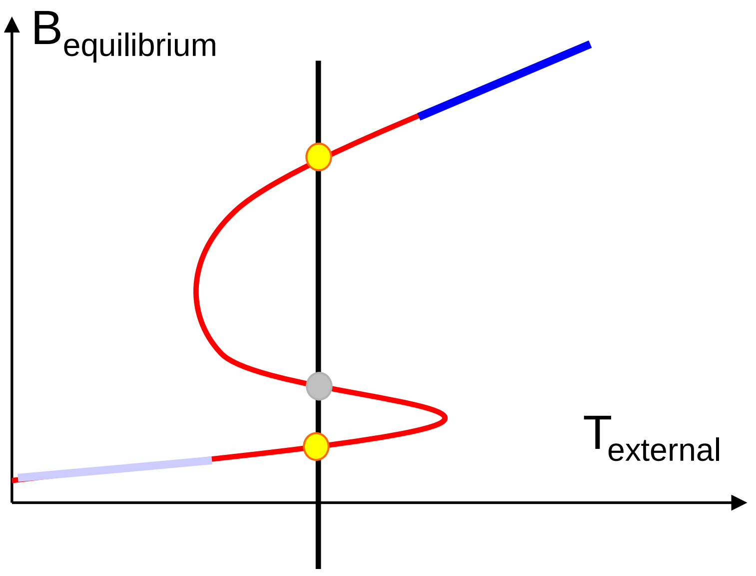
Bistability provides for switching:



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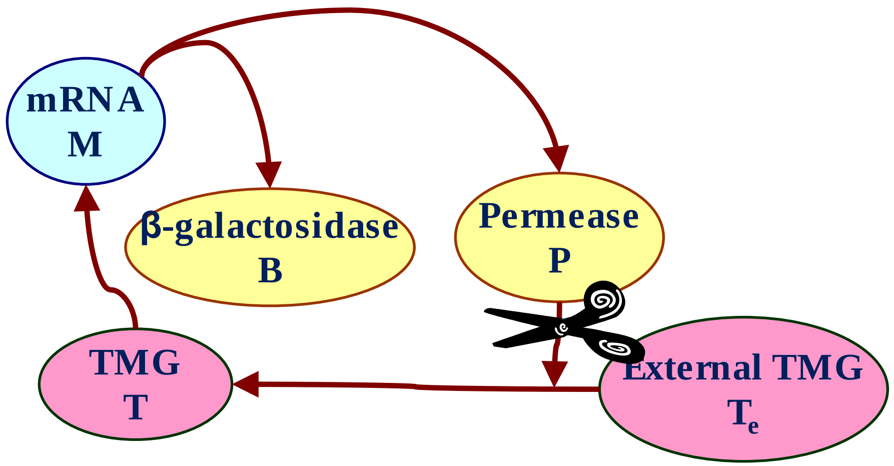


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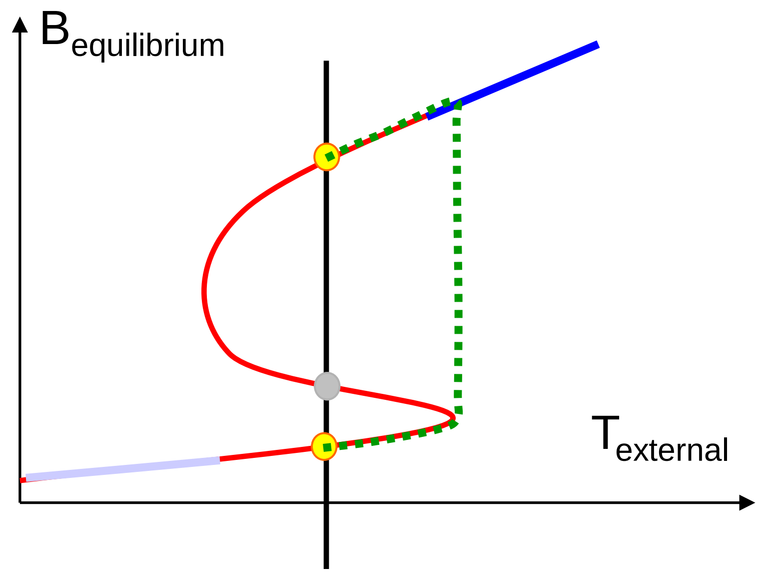


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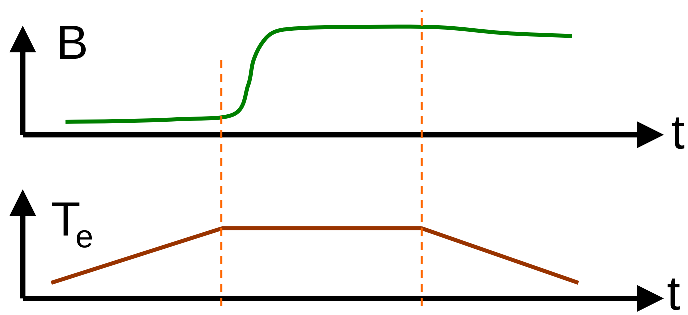
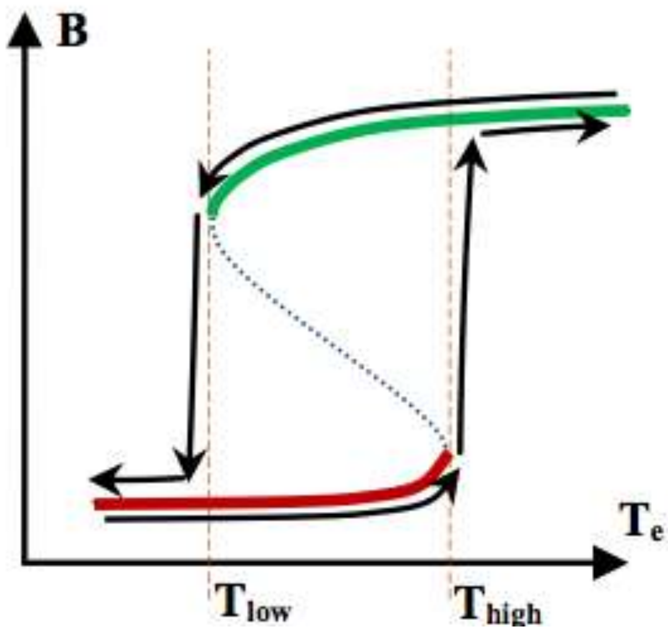
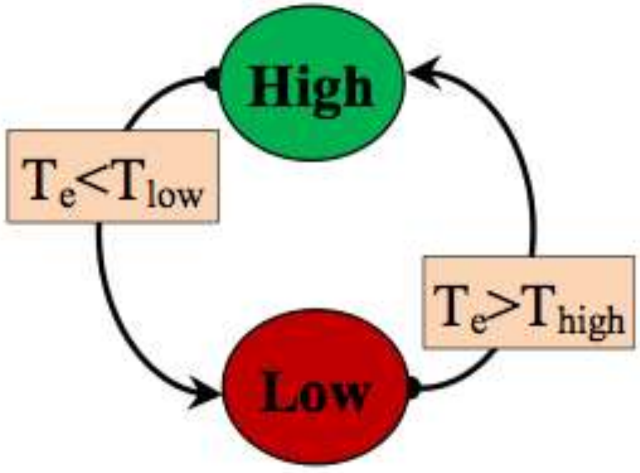


# Abstractions

- A two-state automaton captures the switching behavior
  - The states can be further characterized, individually
  - More often than not, many details are not important as far as the rest of the system is concerned



# Lac system, stochastic model

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The ODE description is not satisfactory:

- once a stable state is attained, the system (cell) should stay there indefinitely
- experimental results show spontaneous transitions and coexistence of two states

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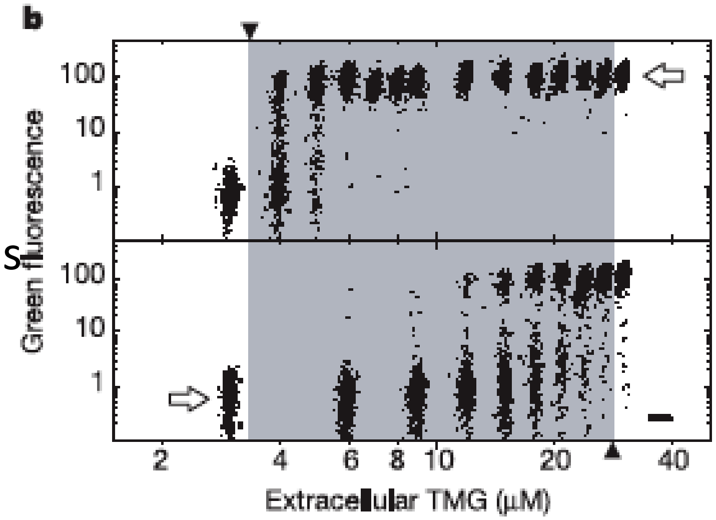

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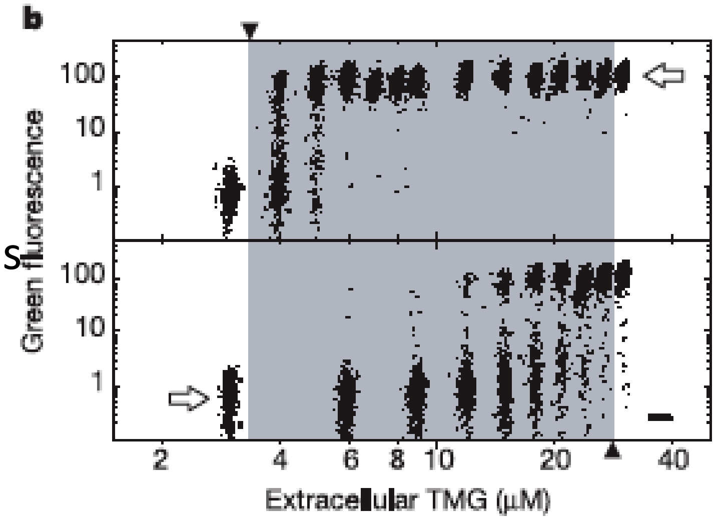
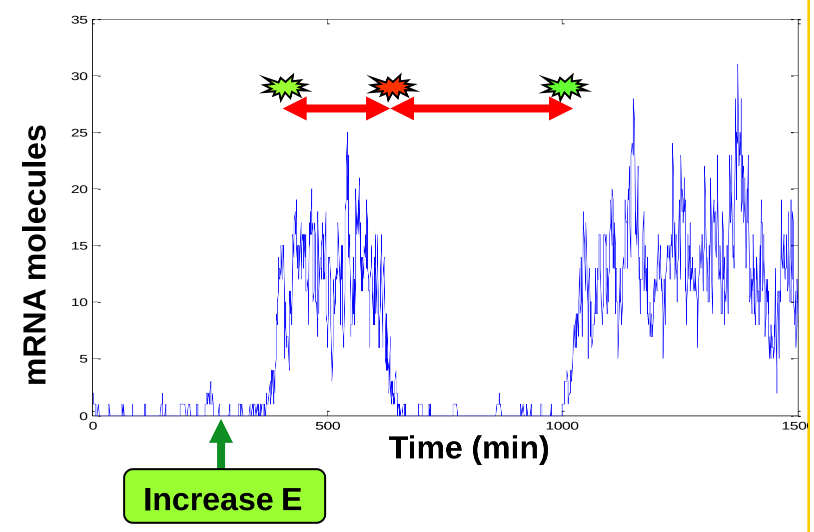



Discrepancy due to small molecule count:

- first-principles stochastic simulations predict spontaneous transitions



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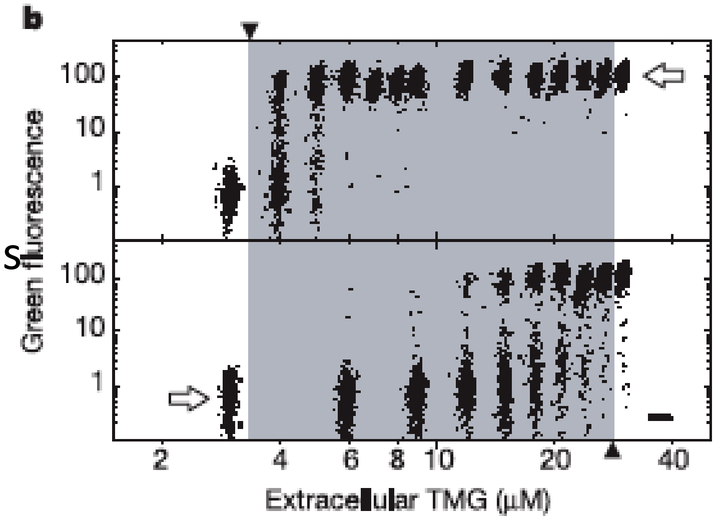


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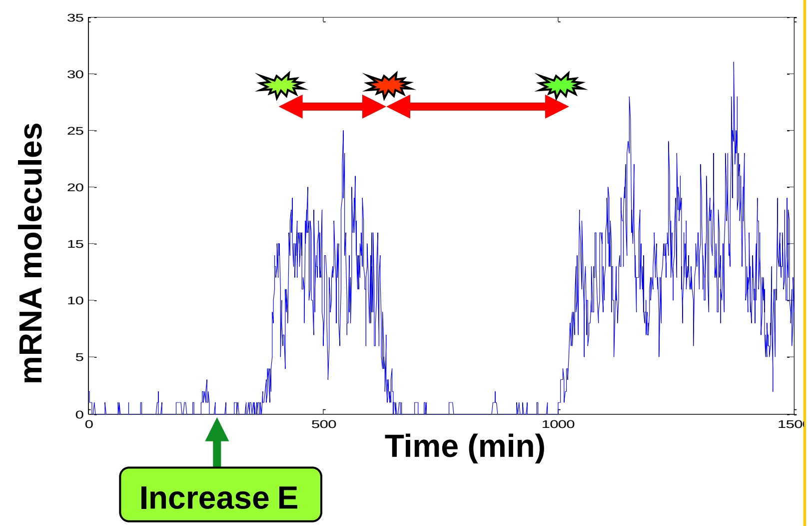
More efficient 'mixed' simulations:

- can perform aggregate simulations
- equilibrium distributions
- compute transition rates

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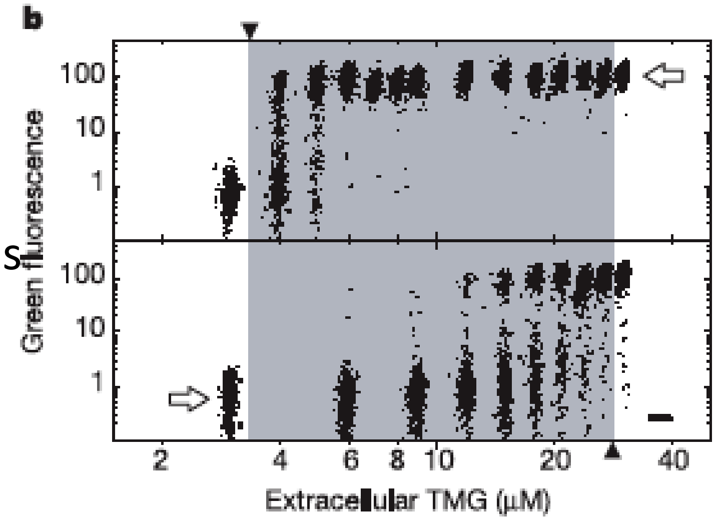


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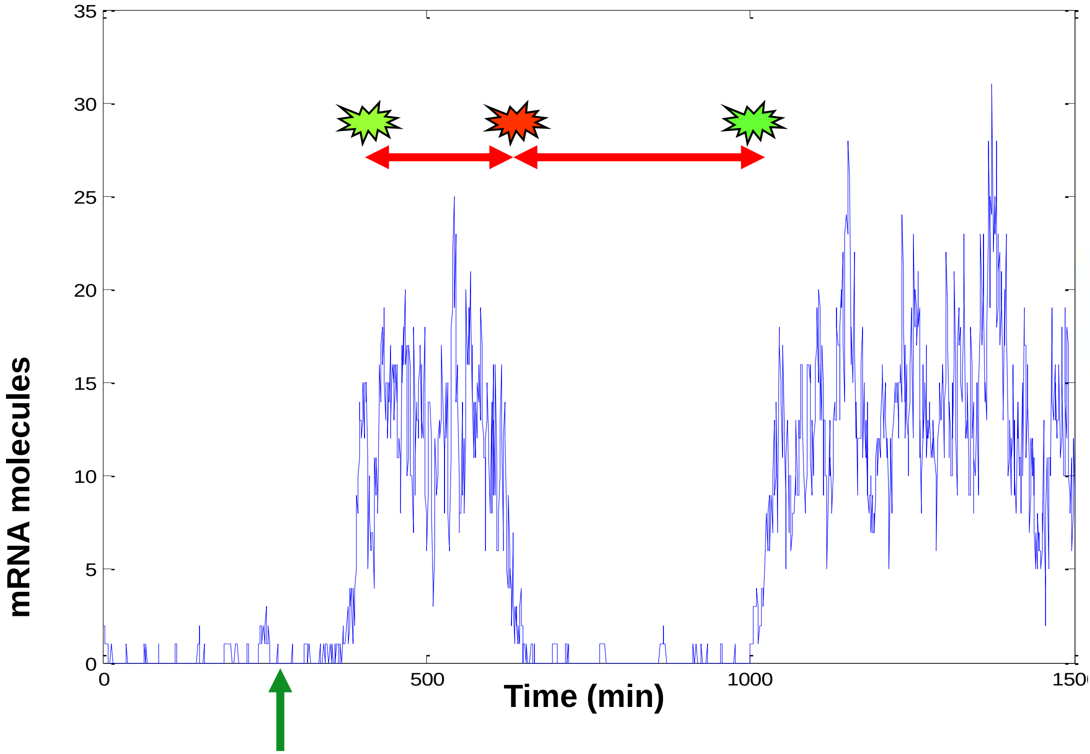
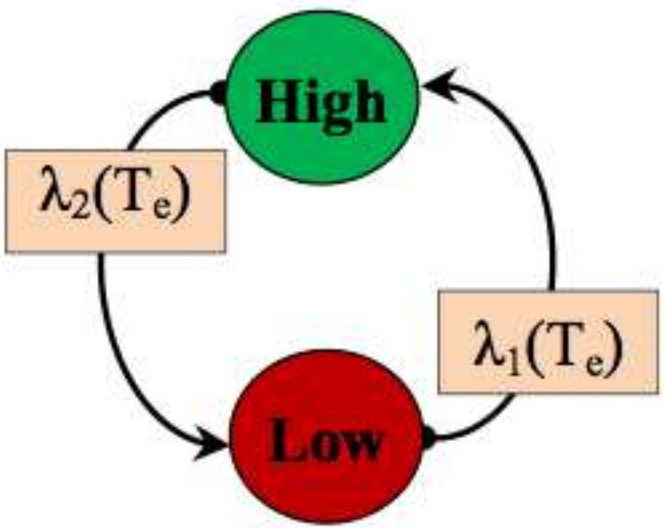
# A stochastic abstraction

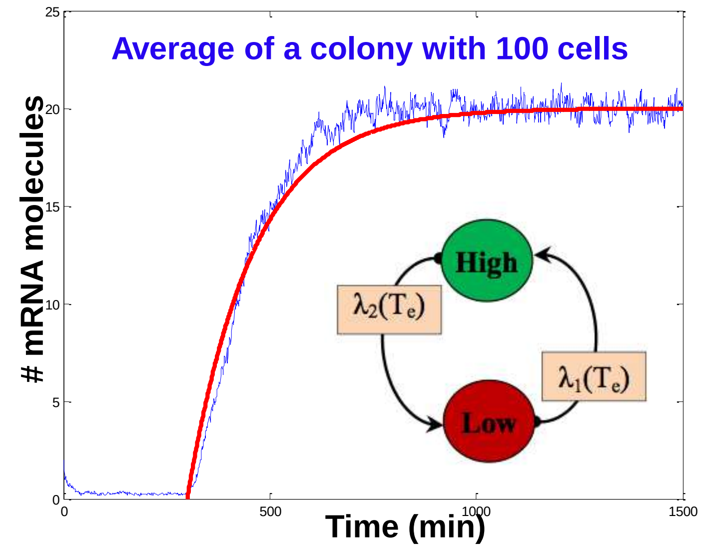
- For intermediate values of $T_e$ there is a quantifiable stochastic switching rate
- Stochastic transitions occur in addition to the deterministic switching triggered by extreme values of $T_e$



# Lac system

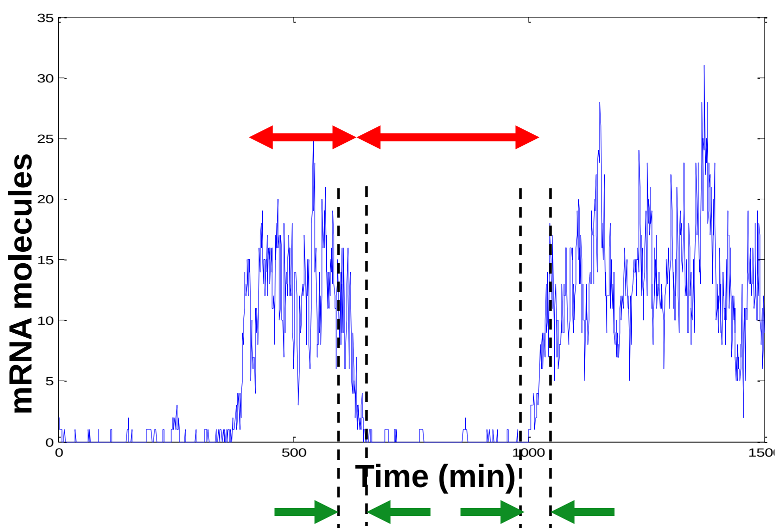
System well described by an abstraction:
- two-state Markov chain model
- transition rates depend on external TMG
- can be computed from the full model




Macroscopic behavior well fitted by this model
- the *timescale of individual transitions* is smaller than the *characteristic time of transition initiation*

*Remaining issue:*

Model parameters are typically fitted to macroscopic measurements
- need to reconcile microscopic and macroscopic model predictions
- possible new insight into *in vitro* vs. *in vivo* parameters

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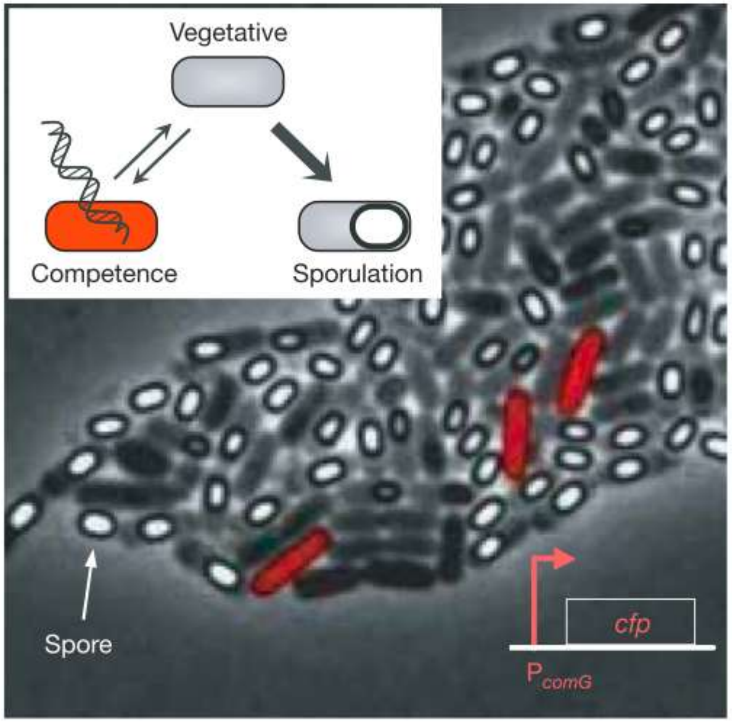
# Lac system

- A classic gene switch
- Simple deterministic dynamics
  - bistability through positive feedback
- Spontaneous transitions due to stochastic effects
  - fluctuations, finite molecule numbers
- Phenomenologically, the two modes coexist
  - the same colony has populations of cells in either state
- Relative population sizes influenced by the characteristic times of the transitions

# Competence in B. subtilis

(based on a paper from the Elowitz lab)




- A two-prong response to nutritional stress
  - Most cells commit to sporulation
  - A small minority (<4%) become competent for DNA uptake
- ComK acts as a "master" transcription factor

Vol 440|23 March 2006|doi:10.1038/nature04588 nature

LETTERS

**An excitable gene regulatory circuit induces transient cellular differentiation**

Gürol M. Süel[1], Jordi Garcia-Ojalvo[2], Louisa M. Liberman[1] & Michael B. Elowitz[1]

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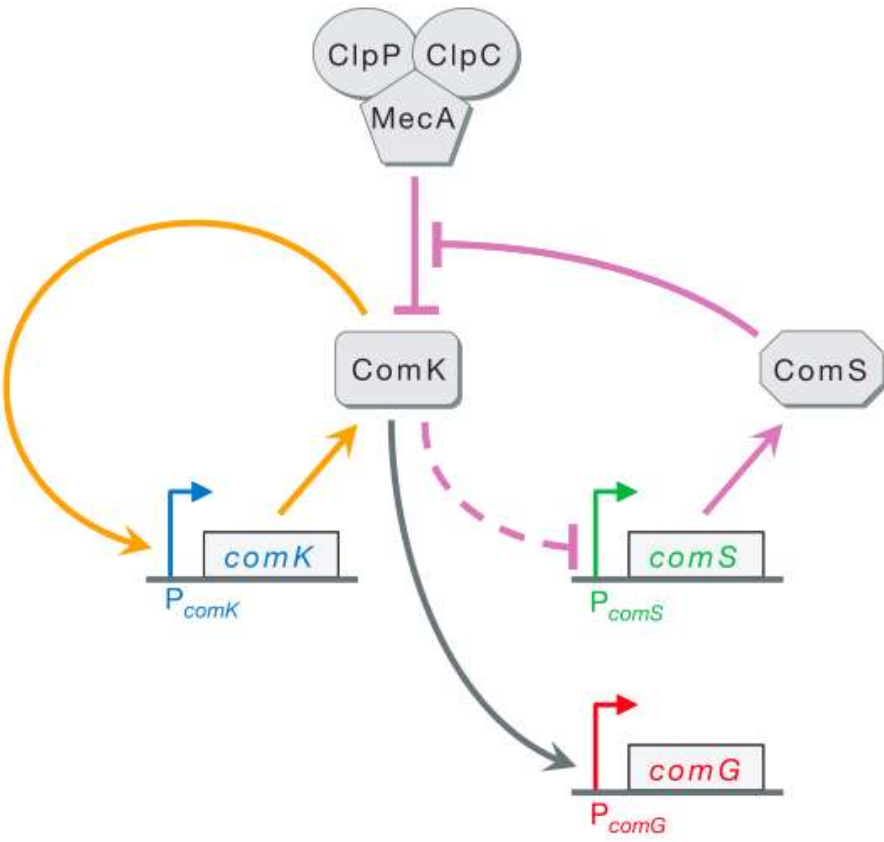
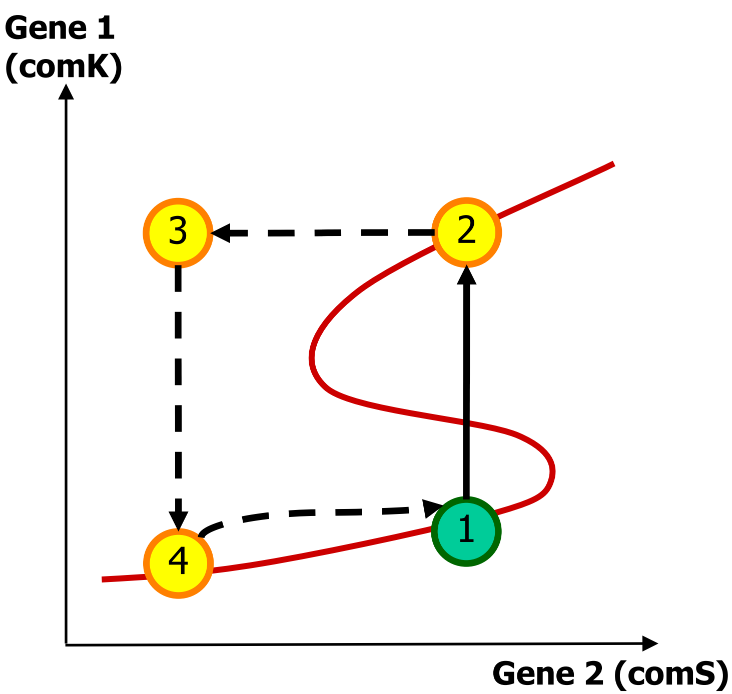


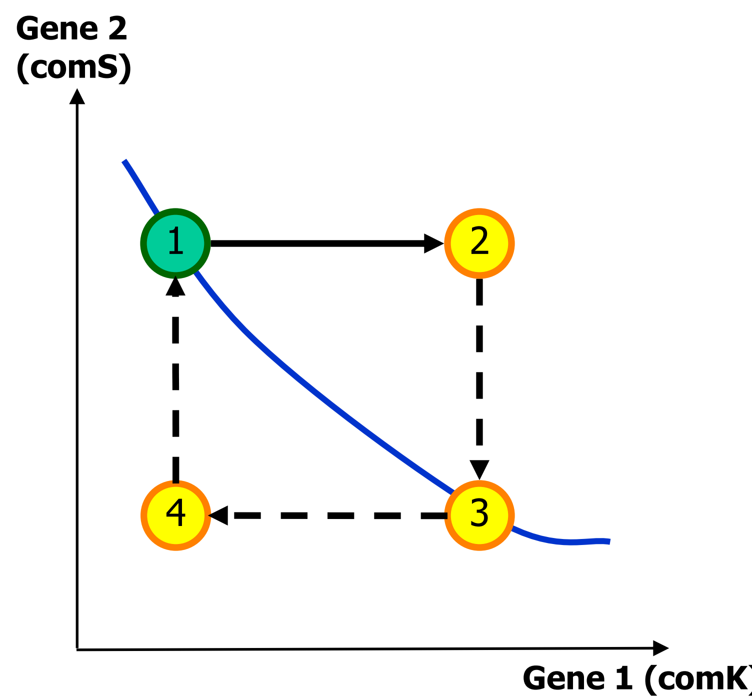

1. *comK* is self-promoting, and is expressed at a basal rate
2. ComK is degraded by MecA
3. ComS competes with MecA, inhibiting ComK degradation
4. *comS* is induced by stress, and is susceptible to noise
5. Overexpression of ComK suppresses *comS*

[Suel et al., Nature, 2006]

# Bistability and slow return

- Two genes with mutual influence:
  - A fluctuation induces Gene 1 (comK)
  - Gene 2 (comS) is inhibited; drops below the threshold for Gene 1
  - Gene 1 returns to its low state, and Gene 2 slowly increases



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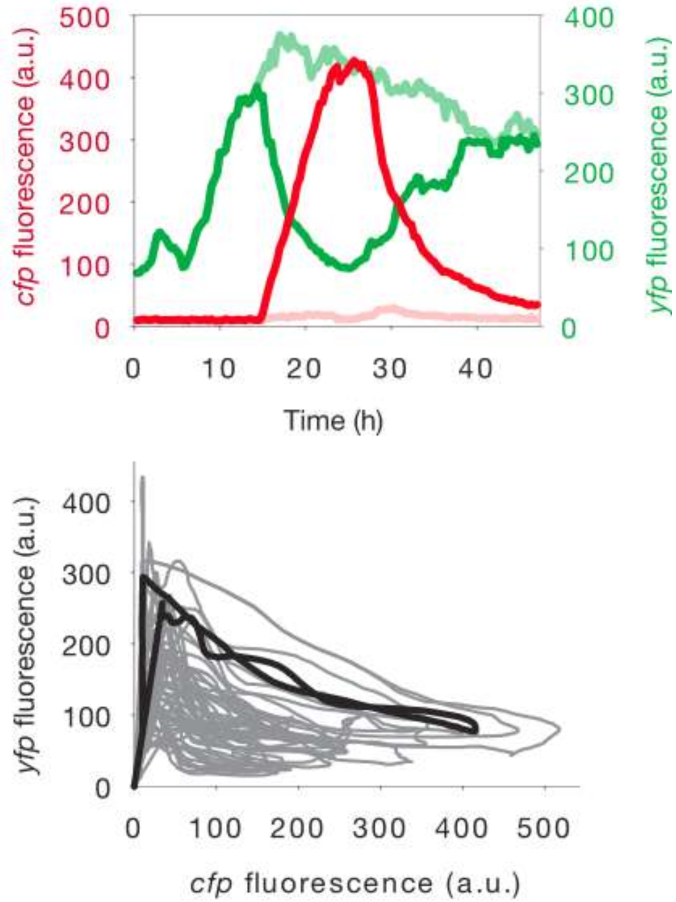



ComS (red) and ComK (green) activities during a competence event

[From Suel et al., Nature, 2006]

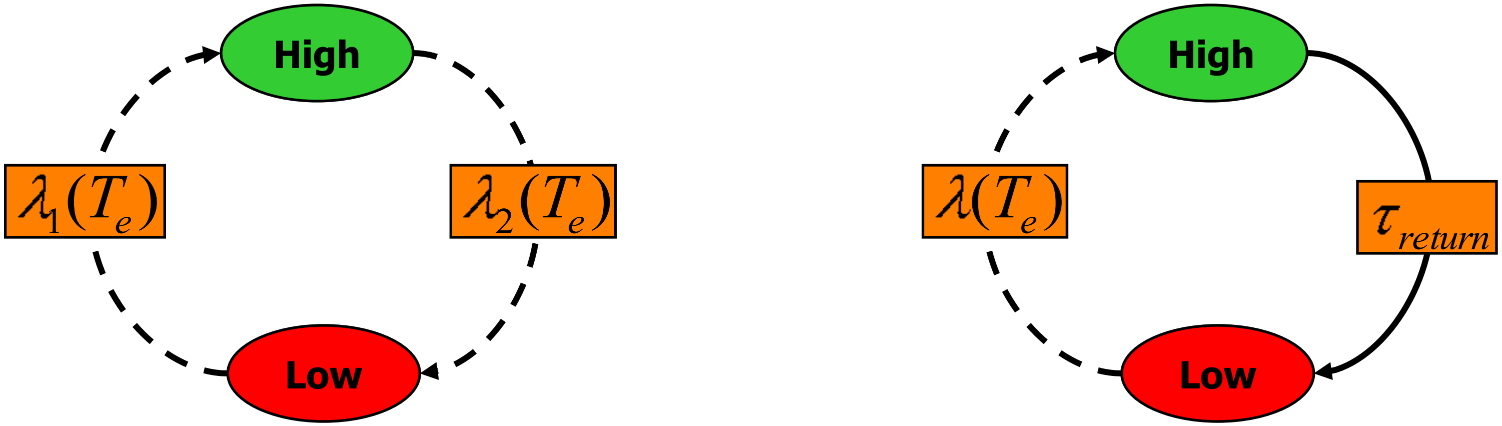
- A fluctuation in **ComS** blocks the degradation of **ComK**
- Increased ComK induces *comK* and the module "flips" into the high mode
- Eventually, the high level of ComK suppresses *comS*
- Lack of ComS leads to increased degradation of ComK
- *comK* "flips" back into the low mode

# The competence example

- Two phenotypes, with identifiable roles in the survival of the species
- Entry into competence is triggered stochastically, similarly to "spontaneous induction" in the *lac* system.
- However, exit from competence is deterministic*;* it is guaranteed by the dynamics of the network
- Even though a bistability motif is present (self-promotion of *comK*), the system is **not** bistable

# A different abstraction

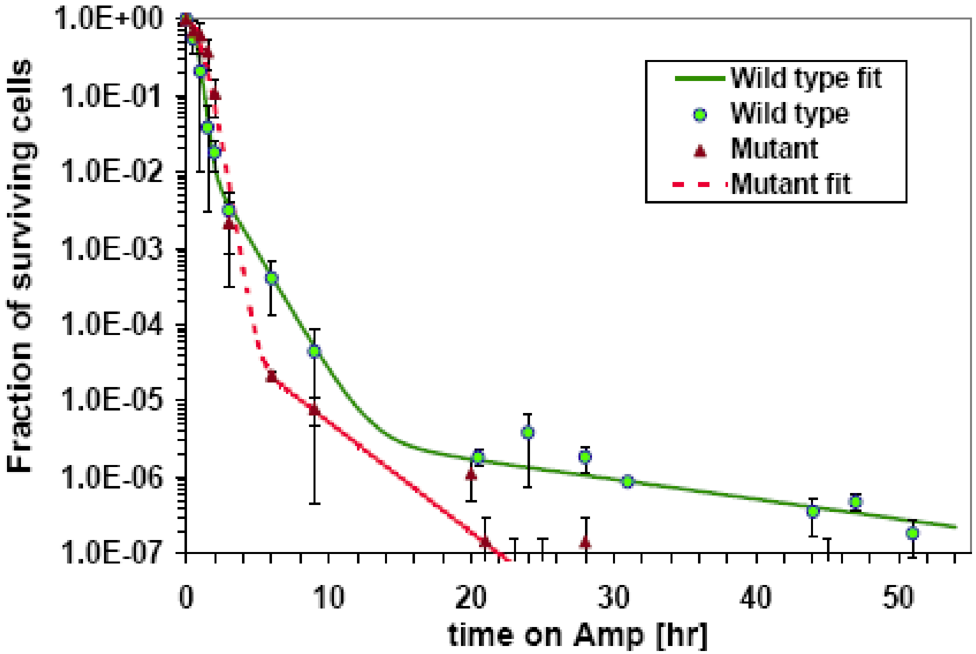
- Only one steady state and a transient
  - Stochastic transition in one direction
  - Deterministic trajectory on the way back
- Similar long-term population distributions



# Bacterial persistence

Discovered in the 1940’s during the first large scale administration of antibiotics

- Small fraction survive therapy at a higher rate than the rest of the colony

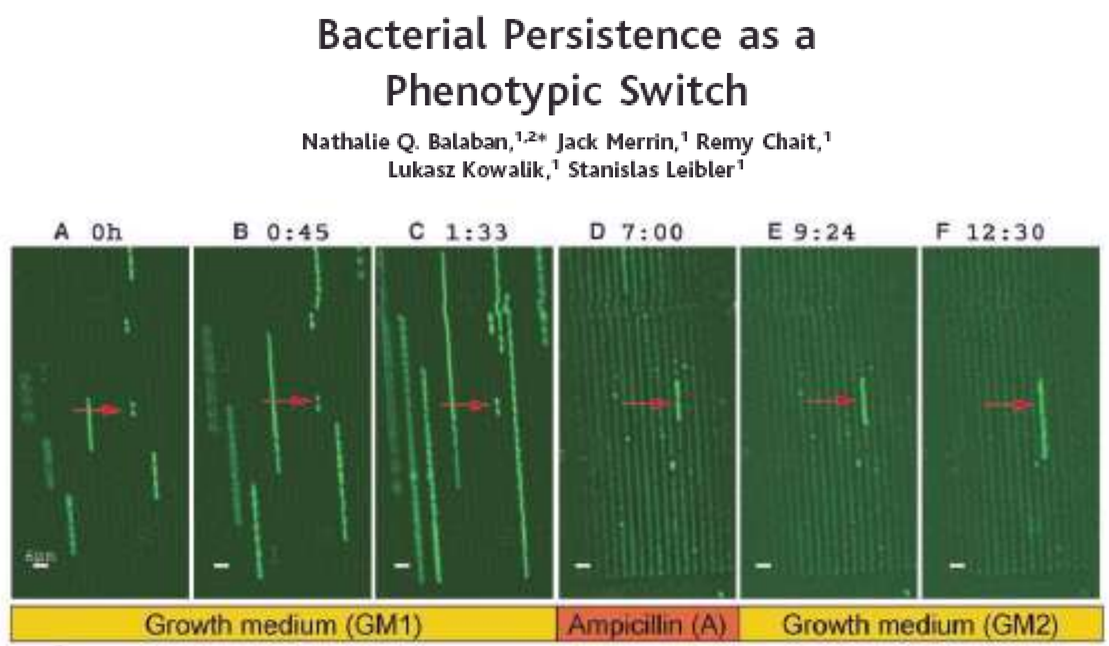



- Persistence opens the door to the emergence of resistant strains
- Persisters are genetically identical to the rest
- They give rise to a colony identical to the old one

# Bacterial persistence

- Persisters are non-growing cells
  - Some are generated during stationary phase
  - There is spontaneous persister generation

- Persistence is an alternative phenotype
  - "Hedging strategy"
- Mechanism not well understood
- Likely an example of spontaneous entry and slow, deterministic return to growth




[From Balaban et al., Science, 2004]

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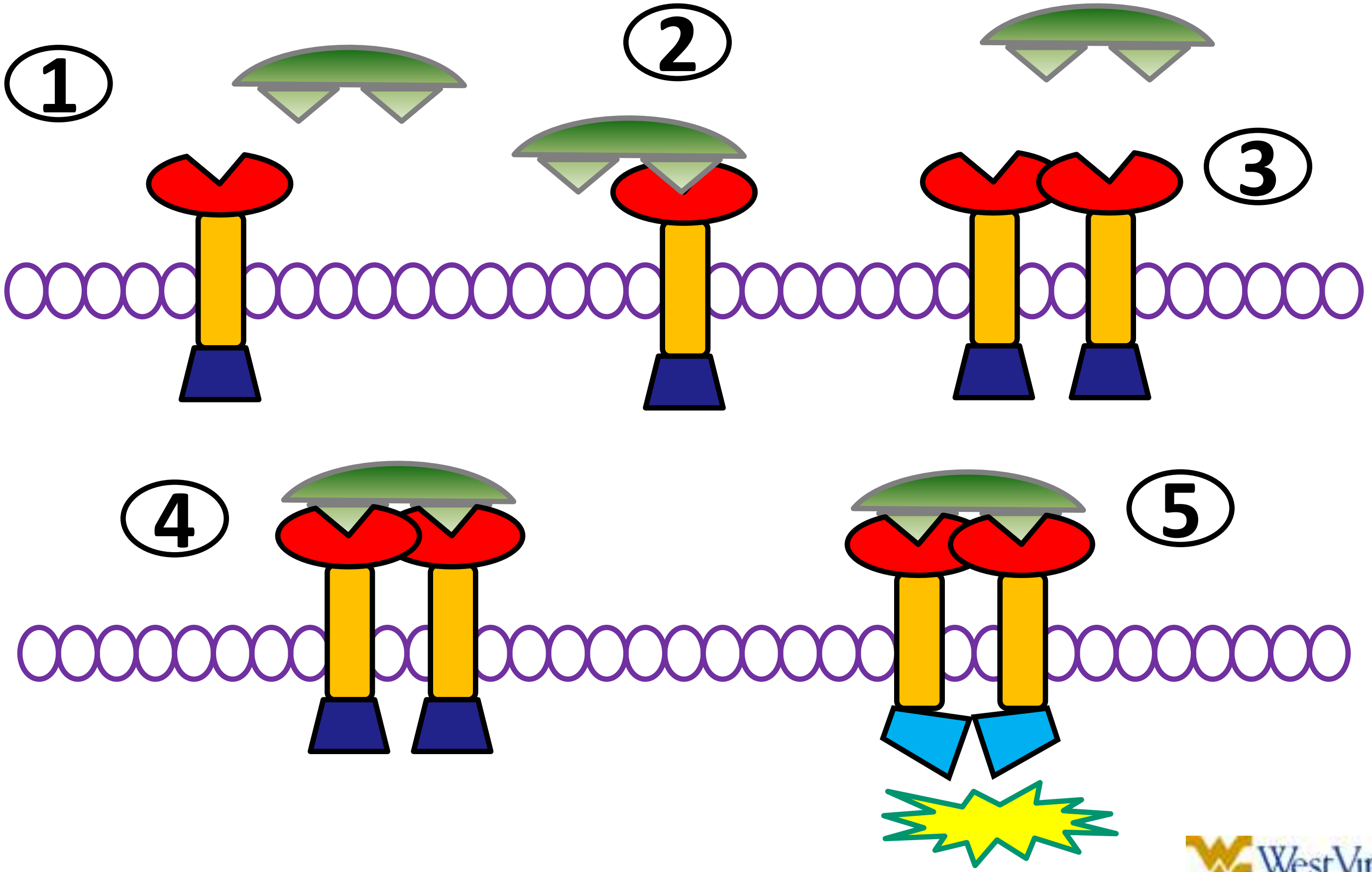
# Spatial effects in cell signaling

- Cells must coordinate in multicellular organisms
  - This is achieved through signaling; signals are special substances
  - Specialized receptors on the cell membrane, some inside the cell

- Receptor tyrosine kinase (RTK) receptors have to dimerize in order to signal
  - These are membrane receptors; they can move more or less freely on the membrane
  - Dimerization is more likely if the receptors are located in high density patches, rather then being uniformly distributed
  - Such patches have been observed; the mechanism behind their formation is unclear
- Spatial self-organization contributes to the dynamics of signal initiation

# Membrane receptors

- Large molecules which straddle the cell membrane
- Ligand binding and dimerization are required for signal initiation

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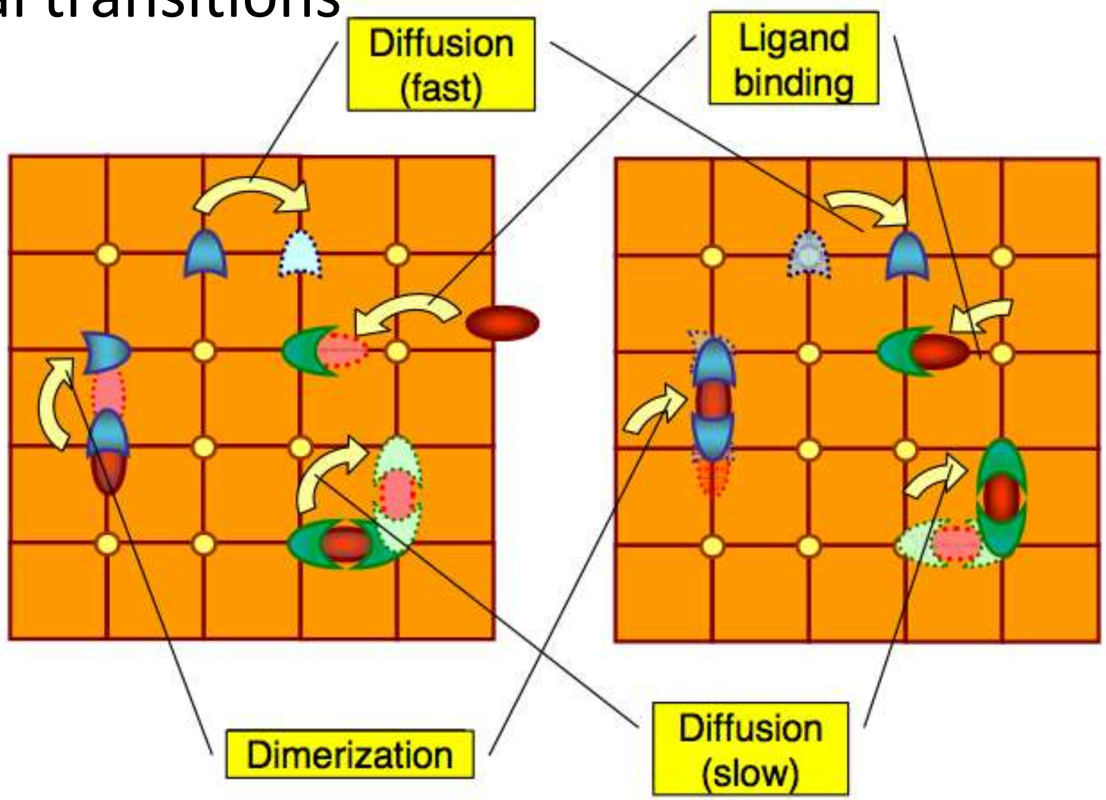
# Spatial Monte-Carlo simulation

- Sometimes the only approach to signal initiation
- Molecules are simulated individually
- The system evolves as a Markov chain with spatial and chemical transitions



# Summary

- Cellular processes can usually be described by ODE-based rate laws
- Two apparently conflicting challenges
  - The complexity of the networks requires simplifications (abstractions)
  - The ODE approach is itself an idealization of a richer underlying phenomenology of stochastic effects and spatial structure
- There are good mathematical methods for abstraction, and good algorithms for simulation

# Acknowledgments


- People (mostly form U.Penn):
  - Harvey Rubin, Vijay Kumar, Junhyong Kim
  - Marcin Imielinski (HMS), Agung Julius (RPI), Selman Sakar
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  - Penn Genomics Frontiers Institute / Commonwealth of Pennsylvania
  - State of West Virginia