

# **Kinetic Model of (p)ppGpp in Growth Regulation and A Possible Mechanism for Bacterial Persistence in the Presence of Antibiotics**

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# Outline

- Model for the Stringent Response
  - Stringent Response and (p)ppGpp
  - Transcription Control
  - Growth Model
  - Role of Lon-activated toxins
- Application: Bacterial Persistence
  - Phenomenology of Persistence in *E.coli*
  - Search for bistability
  - Persistence without bistability?

# Stringent Response and $(p)ppGpp$

- Stringent response: induced by adverse environmental change:
  - Reduced nutrient (amino-acid) availability
  - Antibiotics
  - Many different stress stimuli
  - Similar in many different bacteria, including *M. tuberculosis* and *E. coli*
- Growth rate dramatically reduced
  - Temporary or permanent growth arrest
- Re-programming of transcription for survival
  - Growth related genes (stable RNA, RNAP) down-regulated
  - Some other genes (amino-acid synthesis, stationary phase) upregulated
- Mediated by two small molecules,  $(p)ppGpp$ 
  - guanosine 5'-diphosphate 3'-diphosphate ( $ppGpp$ ) and 5'-triphosphate 3'-diphosphate ( $pppGpp$ )
  - $(p)ppGpp$  surges during the stringent response then subsides (Lazzarini and Cashel, 1975)
  - differential regulation is the effect of  $(p)ppGpp$

# Mechanism of differential regulation

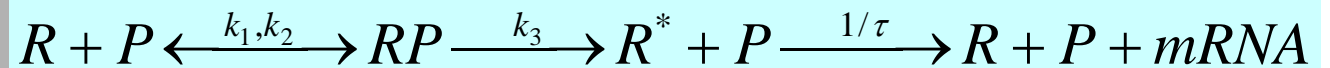
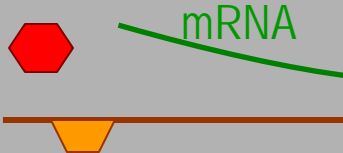
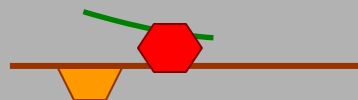
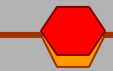
- Transcription kinetics are modified in the presence of  $(p)ppGpp$ 
  - $(p)ppGpp$  attaches to RNA polymerase
  - the complex participates in transcription, but has different kinetic properties
  - the kinetics of transcription vary gradually as the concentration of  $(p)ppGpp$  increases
- The nature and magnitude of specific changes to the kinetics of transcription is subject to some debate
  - Bremer: elongation rate decreases  $\rightarrow$  less free RNAP
  - Gourse: RNAP affinity for stable promoters decreases  $\rightarrow$  more free RNAP

# Mechanism of differential regulation

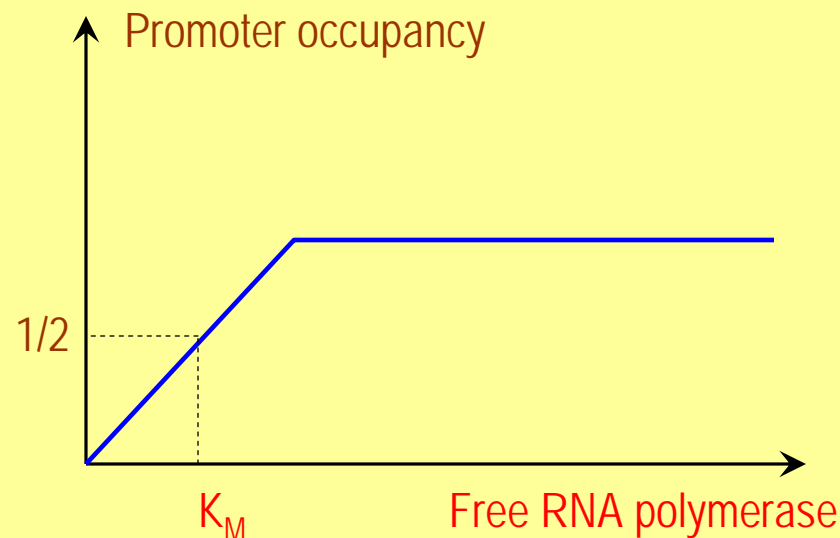
We describe transcription kinetics in terms of effective parameters

- ◆ Transcription depends on free RNAP through promoter occupancy.
- ◆ Each promoter has a saturation constant summarizing initiation kinetics

RNA polymerase

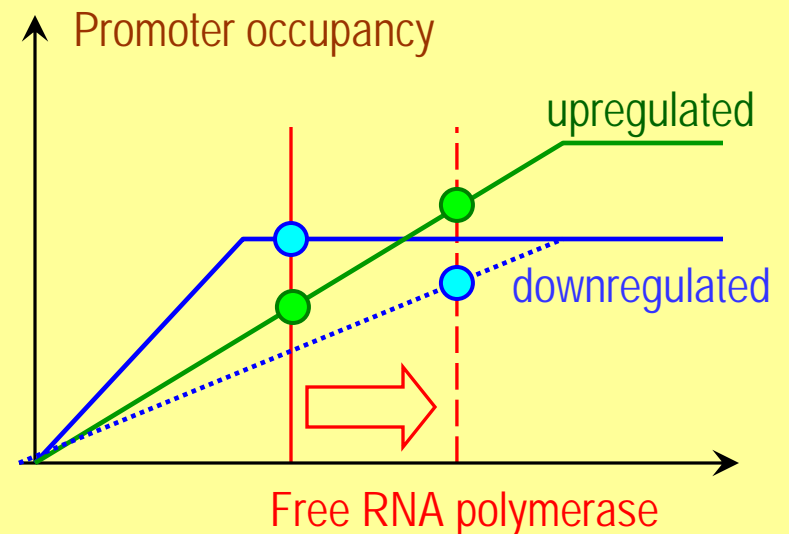
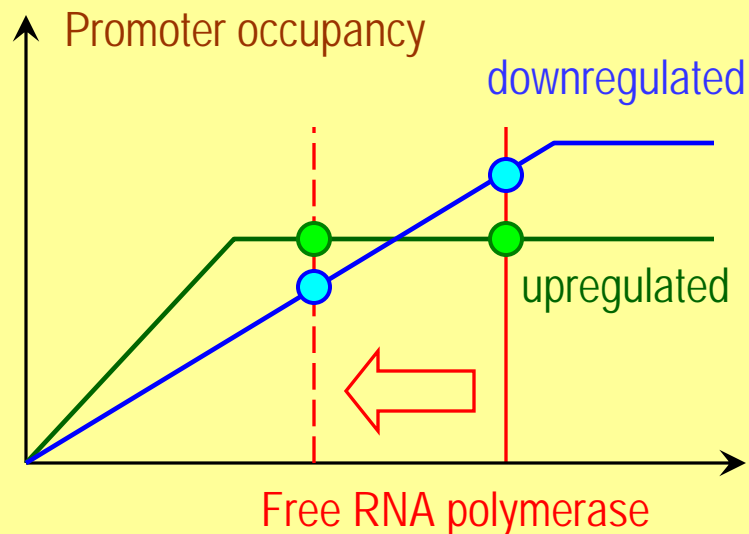


$$K_M = \frac{k_2 + k_3}{k_1}; \quad k_{cat} = k_3$$



# Mechanism of differential regulation

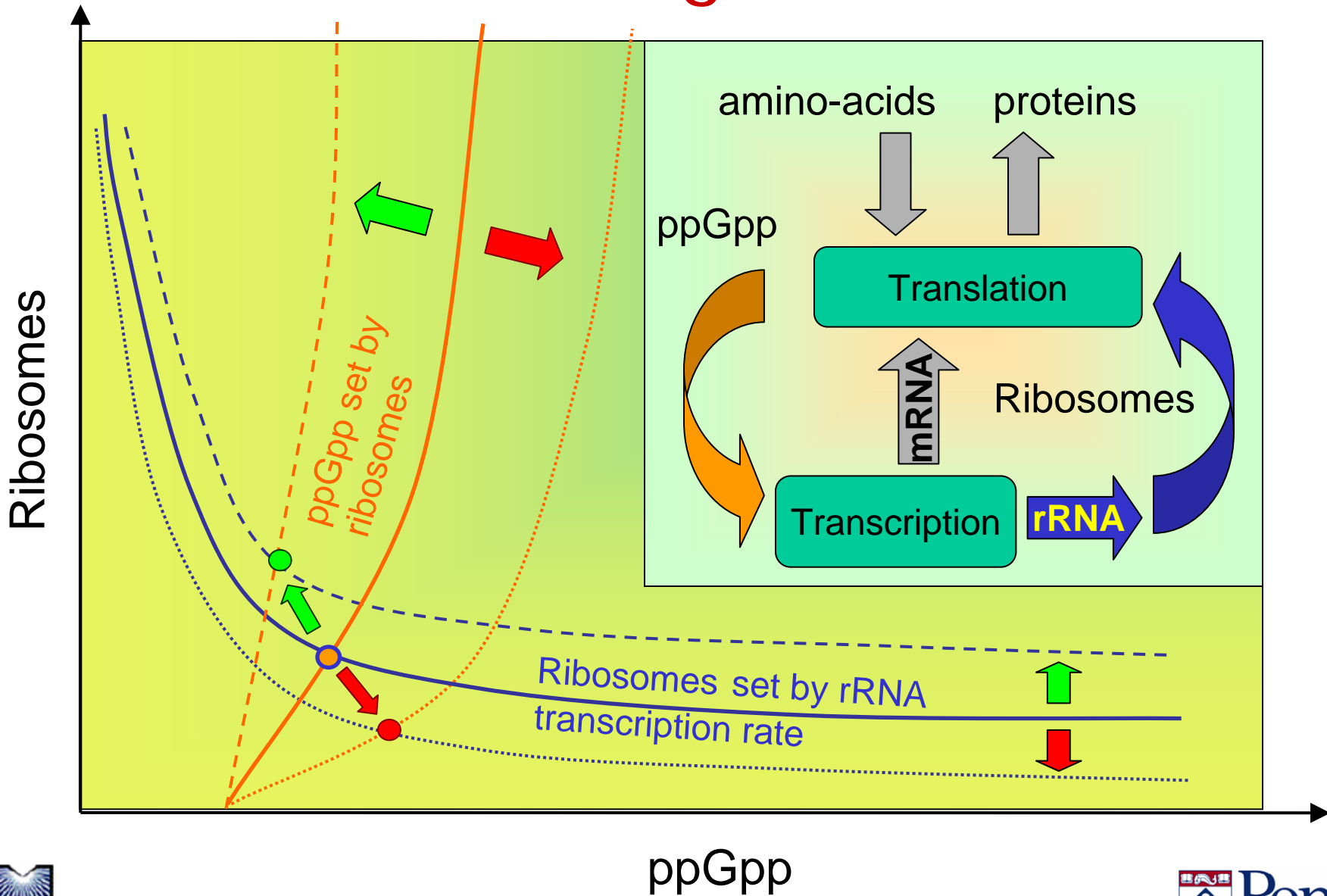
- Transcription time increases
- Free RNAP decreases
- $k_2$  of stable species increases
- Free RNAP increases



# Feedback due to Rel activation

- Two enzymes catalyze reactions involving (p)ppGpp
  - SpoT – heat shock, antibiotics
  - Rel – amino-acid deprivation, growth rate control
- Uncharged tRNA may bind to translating ribosomes – ‘stalled translations’
  - Also called ‘Rel activating complex’ (RAC)
  - Rel probably binds to RAC and is activated
  - (p)ppGpp synthesis increases dramatically
- A negative feedback loop, controlled by the availability of nutrients:
  - (-) amino-acid concentration  $\rightarrow$  (+) tRNA<sup>u</sup>  $\rightarrow$  (+) RAC  $\rightarrow$  (+) (p)ppGpp  $\rightarrow$   
(-) rRNA  $\rightarrow$  (-) ribosomes  $\rightarrow$  (-) RAC

# Mechanism of growth control



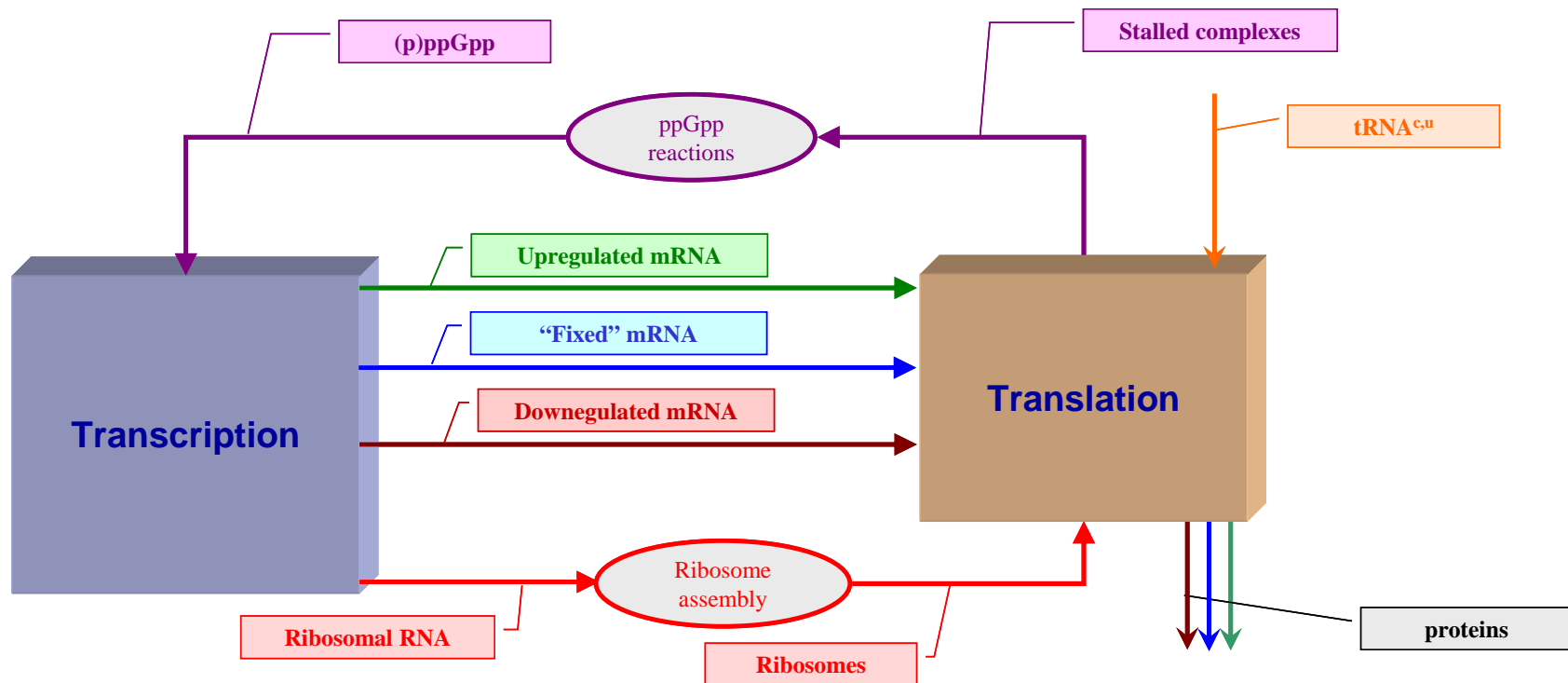
# A wider role for $(p)ppGpp$

- In log phase growth there is a well established correlation between
  - increased  $(p)ppGpp$  concentration
  - downregulation of rRNA transcription
  - reduced growth rate
- Reprogramming of transcription associated with  $(p)ppGpp$  during **stringent response** and during transient growth arrest due to a **diauxic shift** affects almost identical sets of genes (Chang 2002)
- Is the same growth rate control mechanism at work during the stringent response and log phase?



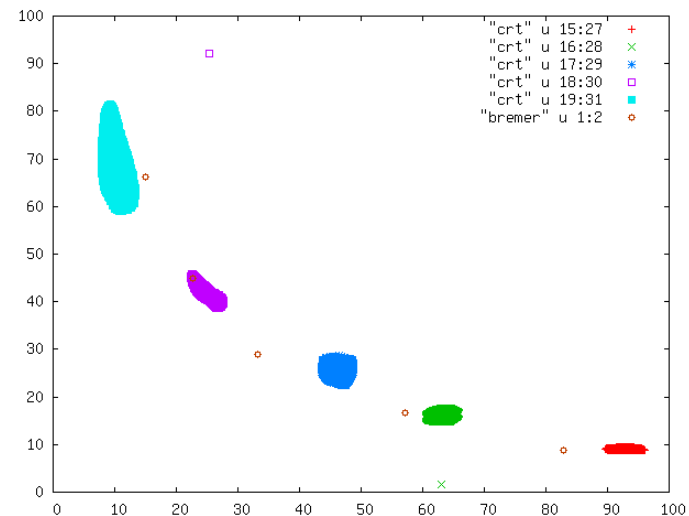
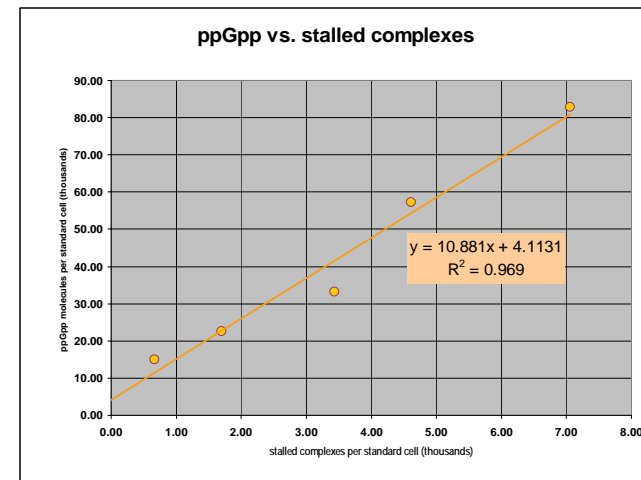
# Model building

- Account for transcription of four promoter groups, translation, (p)ppGpp reactions
- Assume the same mechanism is at work during stringent response and log phase
- Use log-phase growth data from Bremer (1996) for parameter assessment



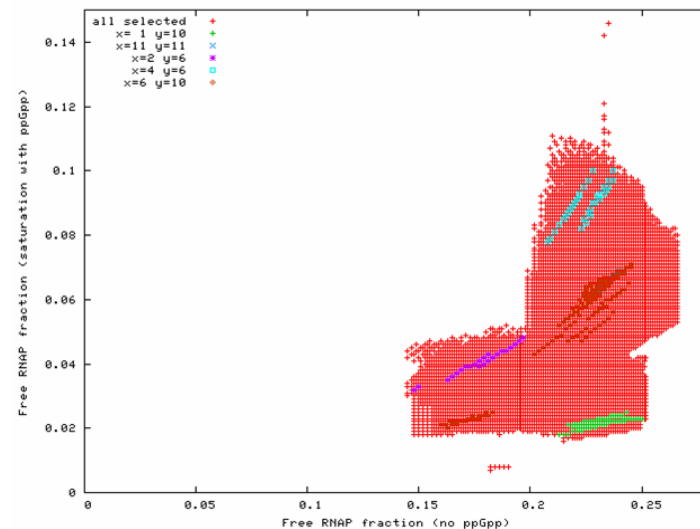
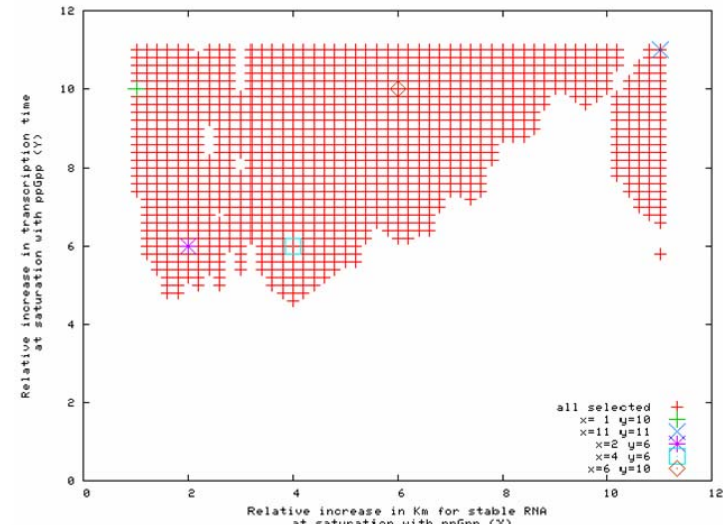
# Parameter estimation

- Promoter counts in the different groups based on genomic data (Cheng 2002)
- Kinetic parameter values from:
  - literature on stringent response
  - direct estimation
  - A group of 8 parameters determined indirectly, by comparing model predictions of RNA transcription rates with the compilation of Bremer (1996)



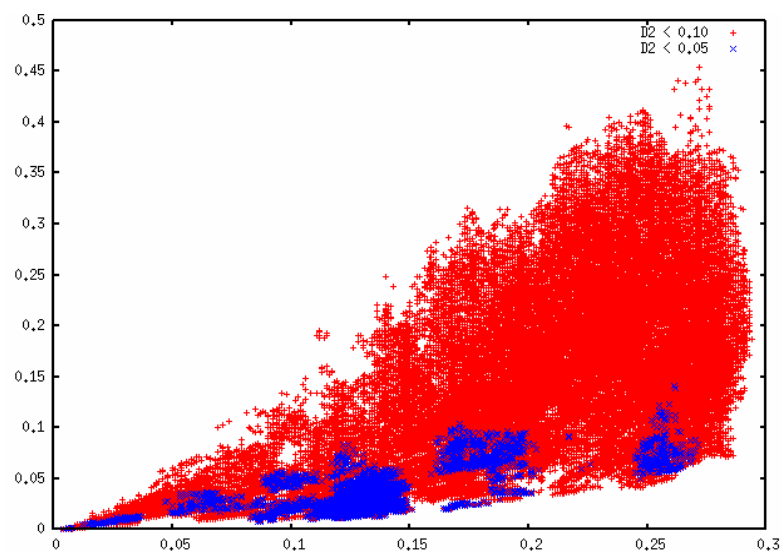
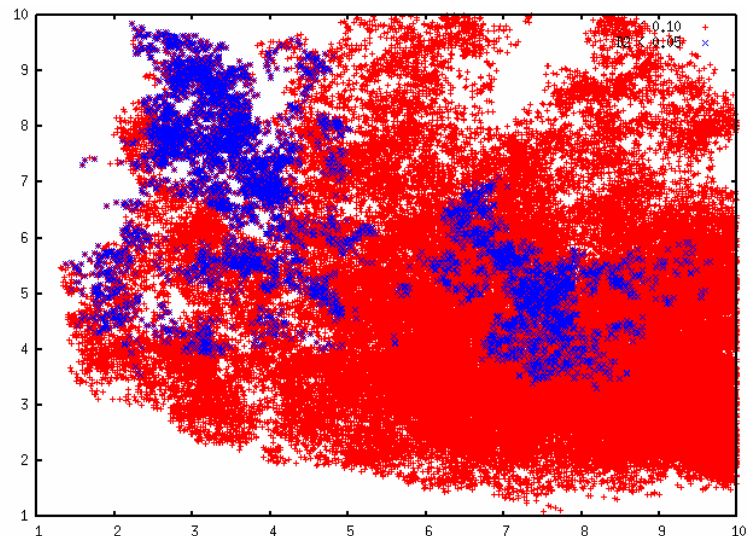
# Parameter assessment using steady state data

- Group of eight parameters related to transcription kinetics whose values are subject to dispute
- Determine by comparing model predictions with log phase data on ppGpp, RNA rates
- Efficient search made possible by the usage of steady state (log phase growth) data for parameter assessment
- Two search methods; collect all parameter sets that provide acceptable fit
  - **Brute force sweep, grid of  $10^8$  values**
  - Metropolis (Monte-Carlo) search
- Many acceptable sets found; uncertainty simply reflects lack of constraining data
- Sets of acceptable parameters can be further culled, by running them on other experimental data

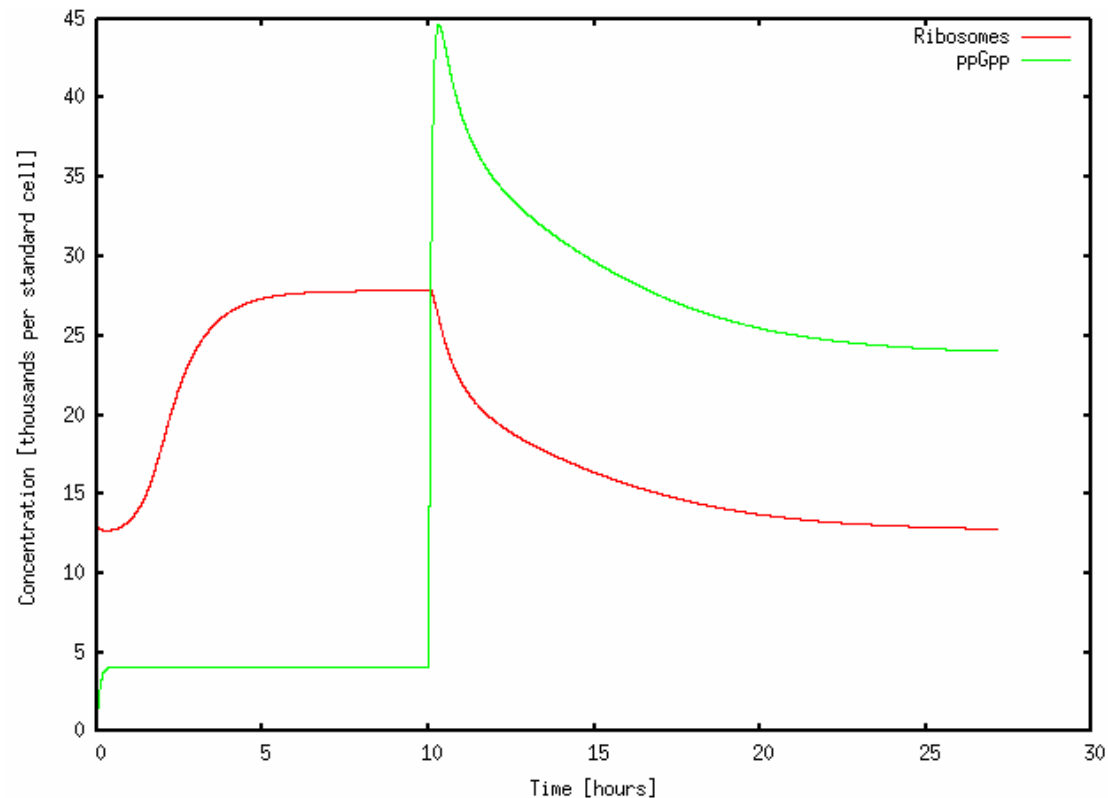


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# Time dependent simulations



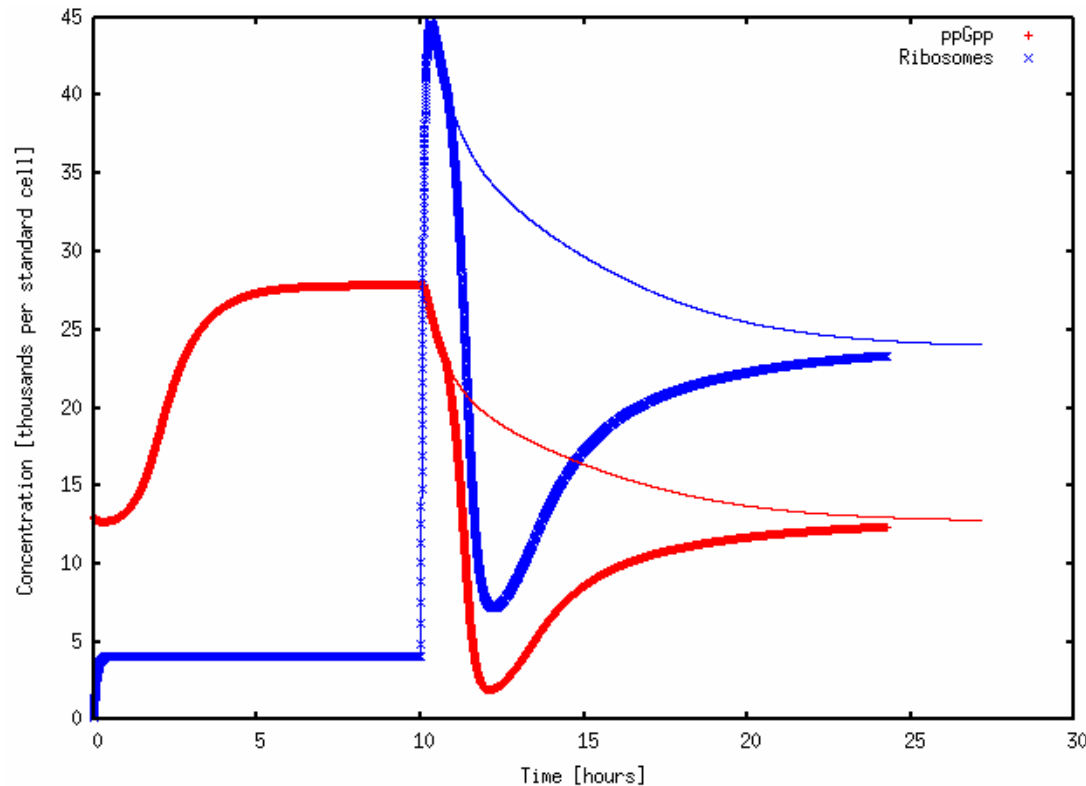
- Simulate nutritional downshifts
- Surge of (p)ppGpp reproduced
- Drop-off is slower than the experimental result
- Need for a mechanism to quickly reduce (p)ppGpp

# The second branch of the stringent response: Lon, RelBE, tmRNA

- RelB and RelE form a classic toxin-antitoxin pair
  - RelB cleaves mRNA at the stop codon; ribosomes translating it are disabled
  - RelE binds RelB, rendering it inactive
  - Other similar TA modules may be involved (mazEF, ...)
- Lon triggers a transient imbalance of RelB/RelE
  - Lon increases the degradation of RelE, creating an excess of RelB;
  - Lon can be triggered by (p)ppGpp
  - Leads to a drop of translation, so RelB eventually also degrades
  - At the end of this cycle, the cell has drastically reduced the number of *active* ribosomes → no more RACs → no more ppGpp
  - Lon/RelBE explains the fast drop in ppGpp following S.R.;
- Ribosomes can be rescued by tmRNA, returning to normal growth
  - Overexpression of RelB leads to 'stasis'; cells can't grow because of a lack of active ribosomes
  - tmRNA brings them back to growth
  - During the initial stringent response, RelB also disables tmRNA



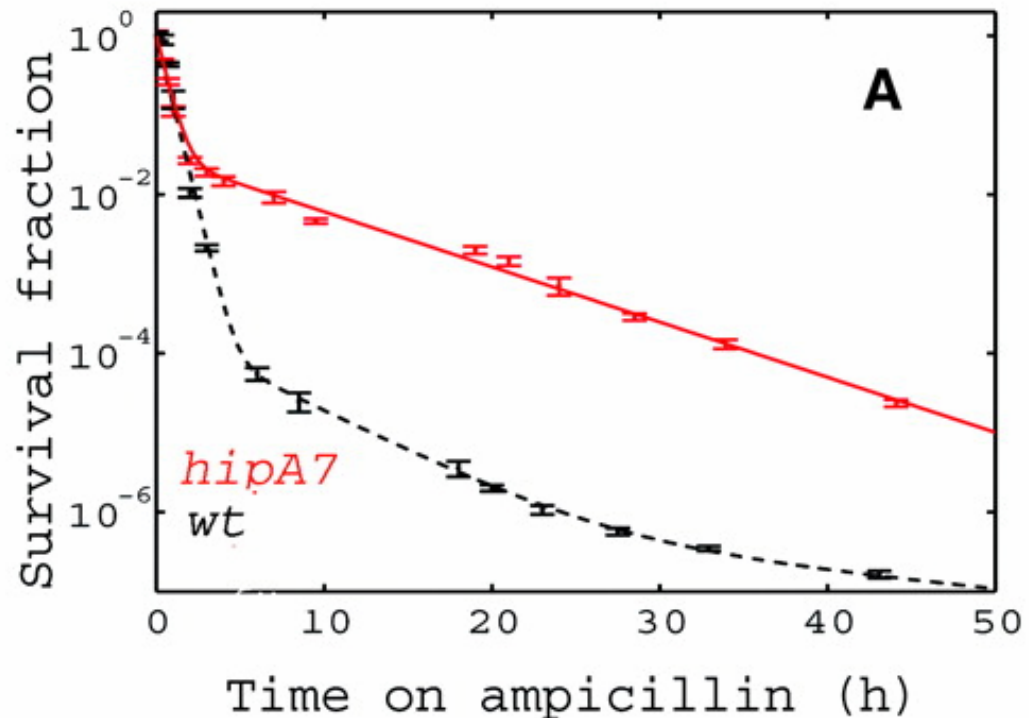
# Inclusion of the Lon/RelBE/tmRNA branch



- Some parameter values readily available
- Crucial parameters unknown, e.g. rate of disabling of ribosomes by RelE
  - Use dimensionless quantities for some of the substances
- Criterion for choice of parameters:
  - Reproduce prompt post-stringent drop-off of (p)ppGpp, through disabling of ribosomes

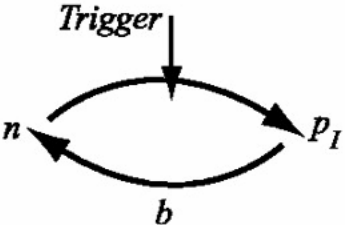
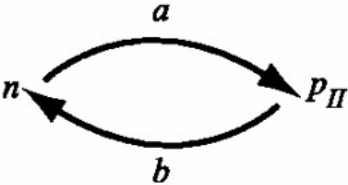
# Application: Bacterial persistence

- Discovered as soon as antibiotics were used
- A fraction of an isogenic population survives antibiotic treatment significantly better than the rest
- If cultured, the surviving fraction gives rise to a population identical to the original one (...)
- Bimodal kill curves
  - Initial steep slope flattened out



# Persistence as a phenotypic switch

- Recent work due to Balaban et al showed that there are two types of persisters:
  - Type I – generated by an external triggering event such as passage through stationary phase
  - Type II – generated *spontaneously* from cells exhibiting 'normal' phenotype

Type I persisters	Type II persisters
	
$\begin{cases} \frac{dp_I}{dt} = -bp_I + \mu_p p_I \\ \frac{dn}{dt} = bp_I + \mu_n n \end{cases} \quad \text{Eq.(1)}$	$\begin{cases} \frac{dn}{dt} = -an + bp_{II} + \mu_n n \\ \frac{dp_{II}}{dt} = an - bp_{II} + \mu_p p_{II} \end{cases} \quad \text{Eq. (2)}$

# Type II persisters

BalabanMovie1.mov

**Growth of *hipQ* bacteria in a microfluidic chamber.** Bacteria are first exposed to growth medium and grow in the narrow grooves. Ampicillin treatment (marked "Amp") results in the massive lysis of cells. After the ampicillin is washed out, persister cells grow and divide. Note that the persister cells are slowly growing before, during and after the ampicillin treatment. Time between consecutive frames: 4 minutes during the first growth medium period and the first two hours of the ampicillin treatment; 11 minutes during the end of the ampicillin treatment and the second growth period. (Balaban et al, Science, 2003)

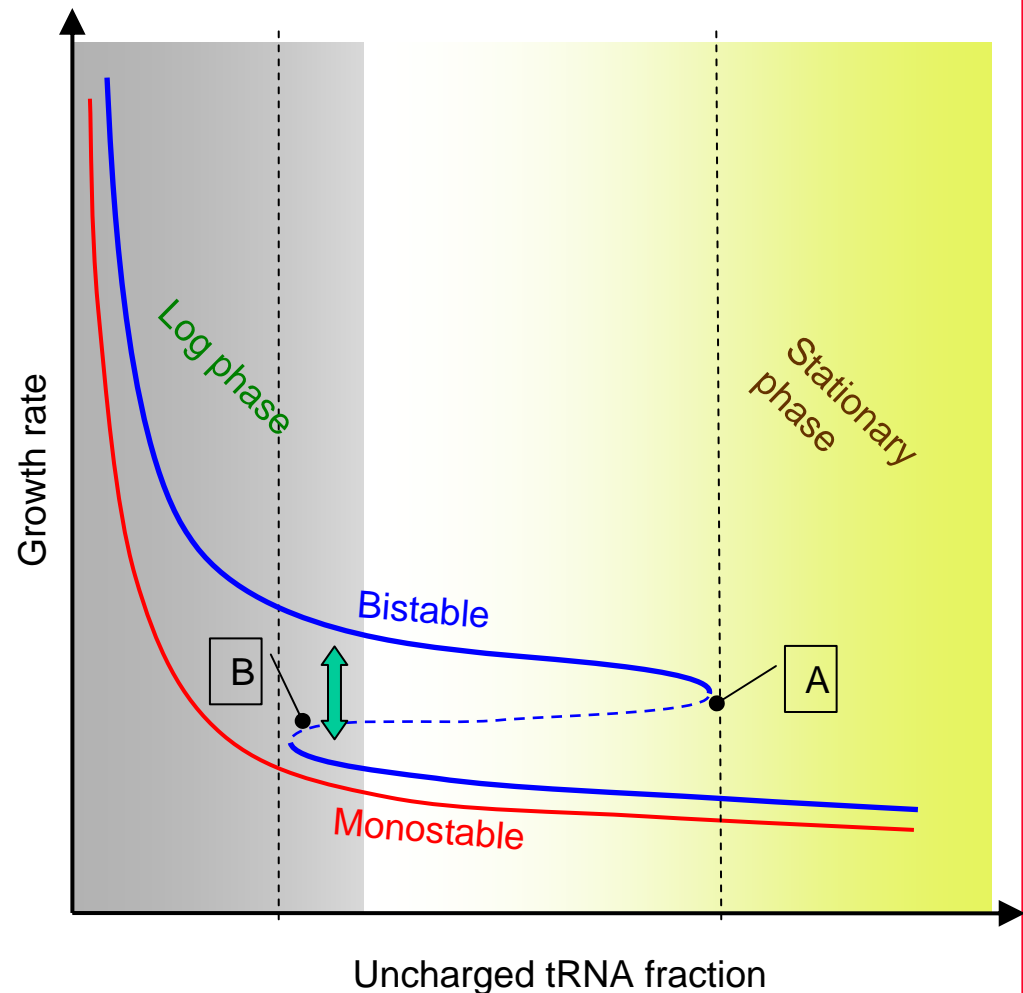


# Persistence as an evolutionary advantage

- Persisters are an alternative phenotype
- Similar to dormancy or stasis
- Since they do not grow, they are less vulnerable
- Presence of multiple phenotypes has an evolutionary advantage in *survival in varying environments*
- Transitions between phenotypes are of *stochastic* nature –
  - Random events, triggered by noise
- What is the underlying molecular mechanism?

# A molecular mechanism for persistence: requirements

- Allows for several steady or quasi-steady states for the same external conditions (such as nutrient availability)
- One state corresponds to normal growth, one to dormancy/persistence
- Rare stochastic transitions are possible between these states

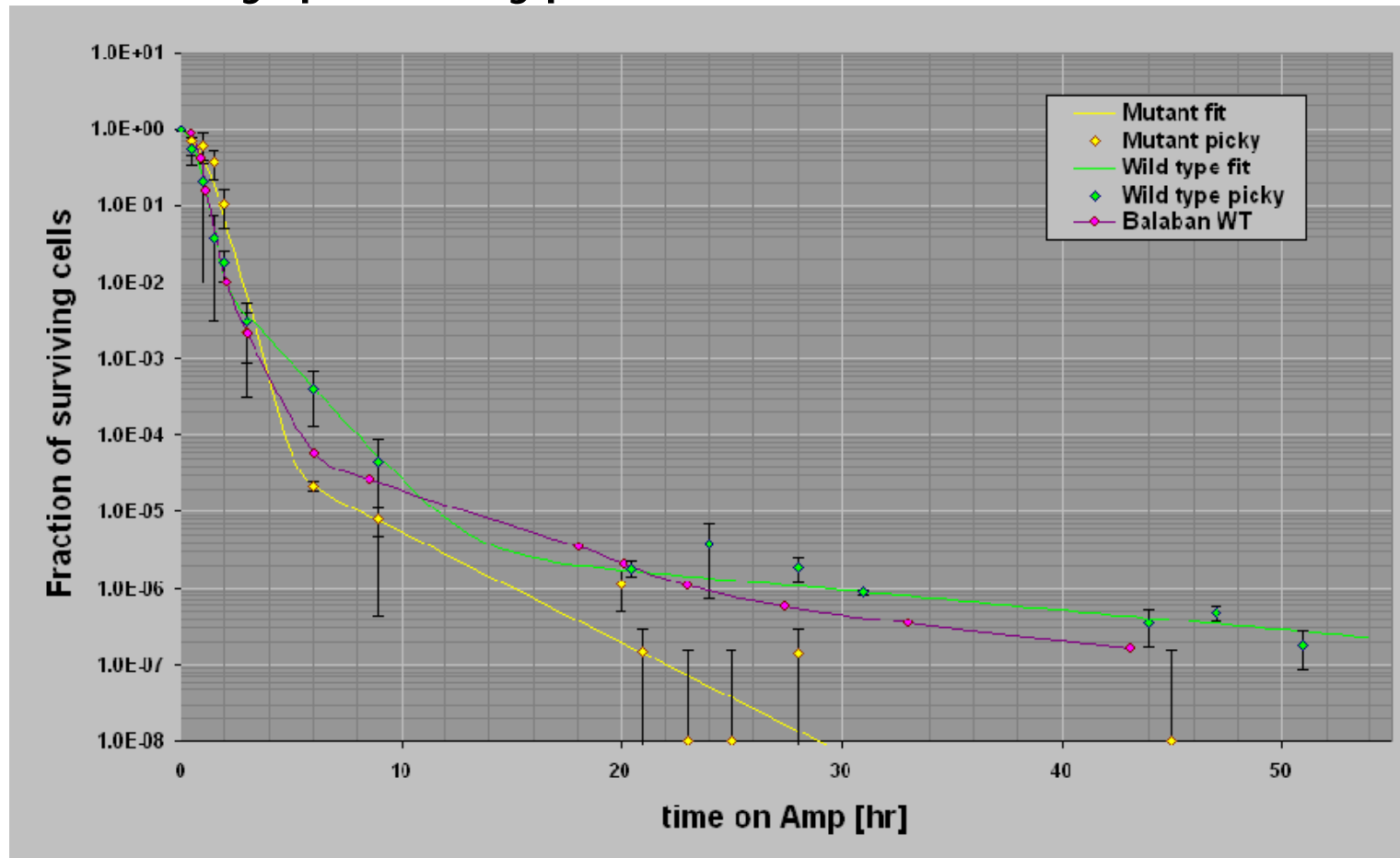


# A possible mechanism: Rel/ppGpp

- Type I persistence is linked to passage through stationary phase
    - ◆ Balaban, 2003
  - ppGpp has a role in the entry into stationary phase
  - Rel knockouts do not exhibit *induced* (Type I) persistence
    - ◆ Korch, 1995
- 
- Can Rel/ppGpp account for growth rate control?
  - Does it allow for growth rate bistability?
  - Is there a mechanism that allows *quick* and *randomly inducible* transitions between the growing and slow states?
  - Does *spontaneous* (Type II) persistence go away when the Rel/ppGpp mechanism is eliminated?

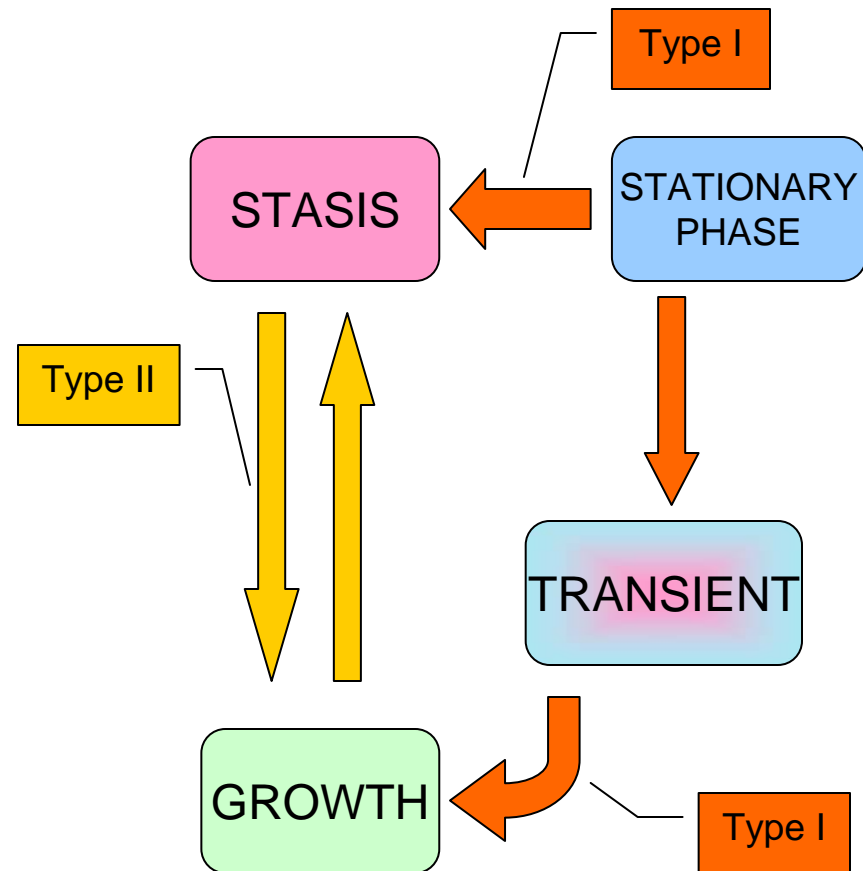
# Experimental results

- Rel/spoT knockouts have severely diminished persistency phenotype:

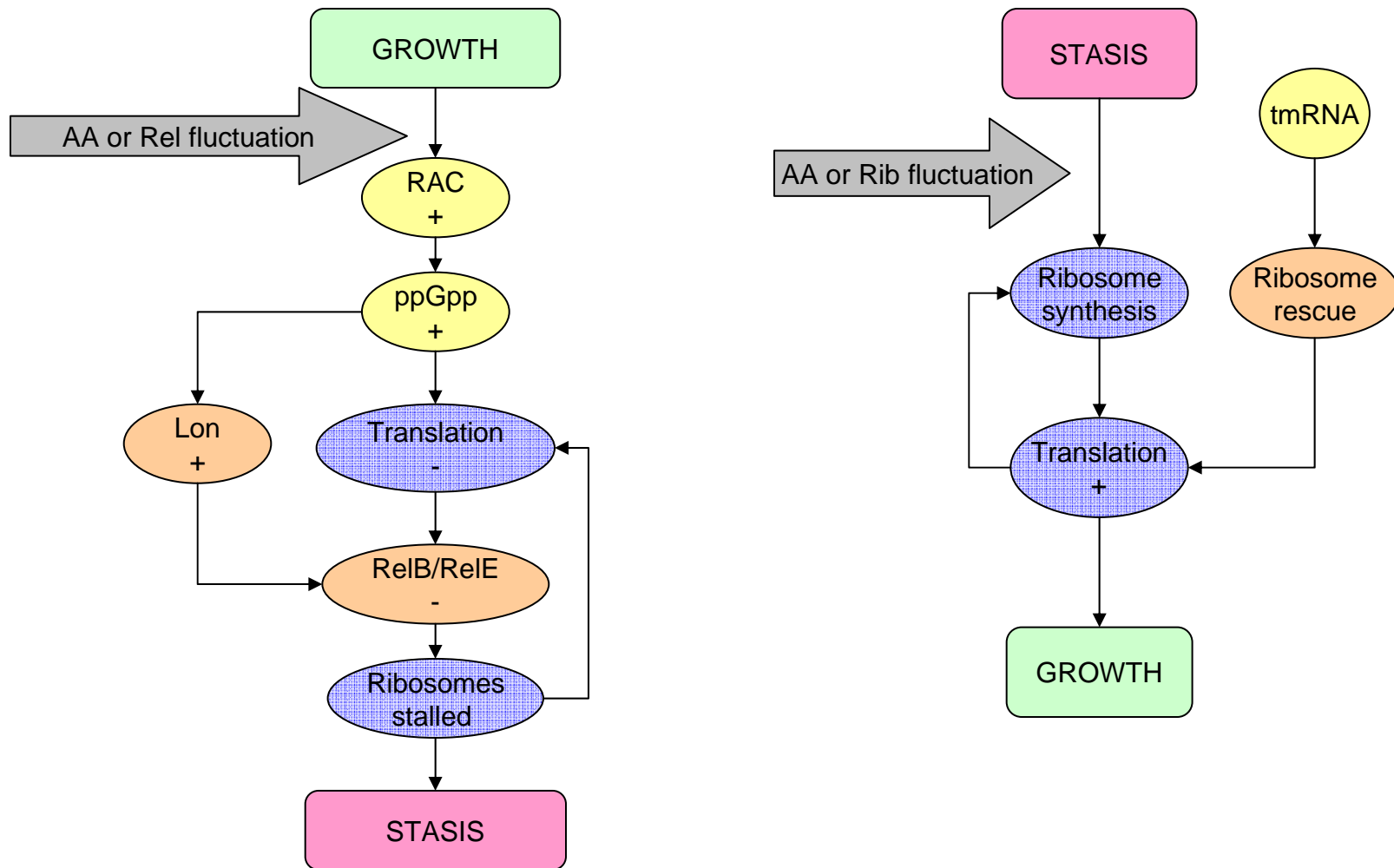


# Hypothesis

- A slow growth state is made possible by the (p)ppGpp growth rate control mechanism
- Transitions into the slow growth state are random events involving activation of RelE following a gratuitous stringent response induced by Rel or amino-acid fluctuations
- Return from slow growth involves stochastic fluctuations which easily take the cell out of the stability region
- The asymmetry is consistent with the very low occurrence of persisters



# Stochastic downward transitions facilitated by Lon

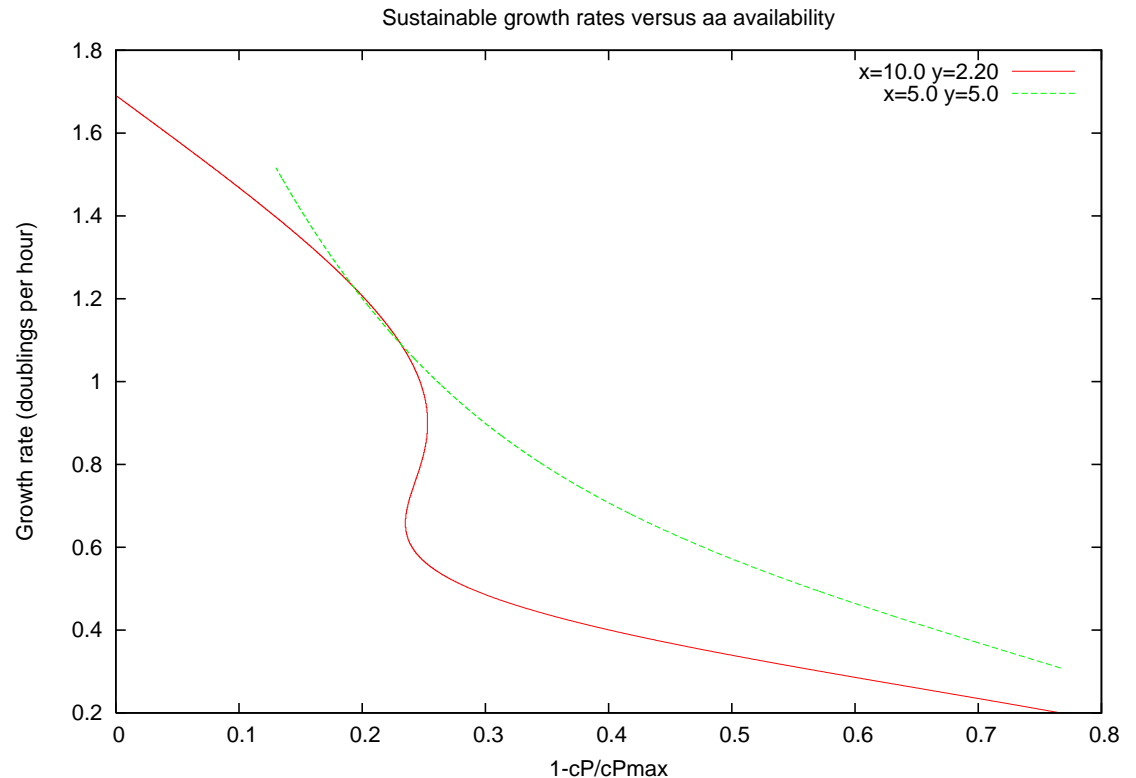


# Bistability

- For now, the parameters that are a good match do not exhibit bistability
- However, slightly modified sets do.
- Many interactions in our mathematical model are simplified

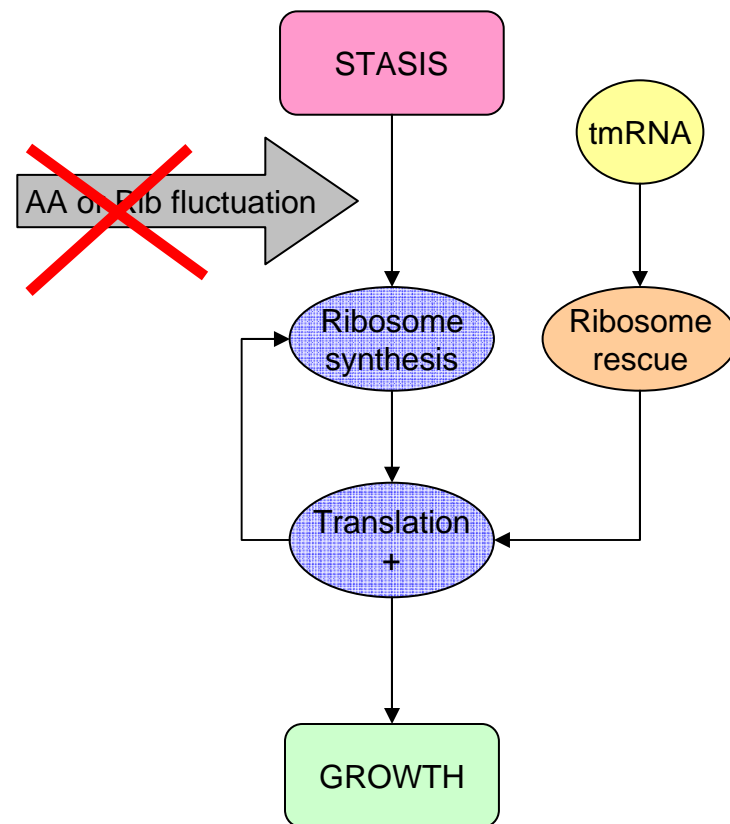
## But is bistability really necessary?

- If the Ion mechanism can be triggered by random fluctuations, the cell can go into a state with very few active ribosomes
- Even if this state is not mathematically stable, it will take a very long time to recover from it
- The role of Rel/ppGpp is still crucial, in defining the landscape of the stable and quasi-stable states



# No need for upward stochastic transitions

- Growth arrest due to a stringent response involves disabling of the cell's ribosomes
- Lack of bistability simply means that a state with few ribosomes is not *mathematically* stable
- Return to growth is possible and deterministic, but slow: even with tmRNA, growth resumes after ½ - 1 hour
- Can a transient slow growth episode be distinguished from a stable slow growing state with a high likelihood of a stochastic exit?



# Summary

## Stringent response/ppGpp:

- The stringent response is part of the stress response system of different types of bacteria
  - *Escherichia coli* – gram negative; *Mycobacterium tuberculosis* – gram positive
- Generic mechanism involves (p)ppGpp, and its effect on translation
  - Mechanism is quite delicate
  - Details still subject to controversy
- Mathematical model of growth rate control mechanism
  - Parameter sets chosen using both log phase and SR data
  - Not conclusive in deciding between different proposed mechanisms
  - Introduction of RelBE/Lon necessary to ensure correct post-stringent behavior

## Persistence:

- Non-induced persistence due to stochastic switching
  - normal, fast growing phenotype
  - dormant, more resilient phenotype
  - Molecular mechanism not yet known
- We propose to explain persistence with Rel/ppGpp
  - ppGpp related to induced persistence, dormancy, growth rate control
  - Our model can accommodate bistability, but not for the best-match parameter sets
  - Experimentally, Rel/spoT knockouts lose persistence
- Bistability not necessary
  - stochastically triggered growth arrest followed by slow return to growth can also explain the observed rare slow growers



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  - Vijay Kumar
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