Bacterial persistence in the presence of antibiotics

Harvey Rubin, Adam Halasz, Maria Maryanovich, Michael Buckstein, Marcin Imielinski, Vijay Kumar

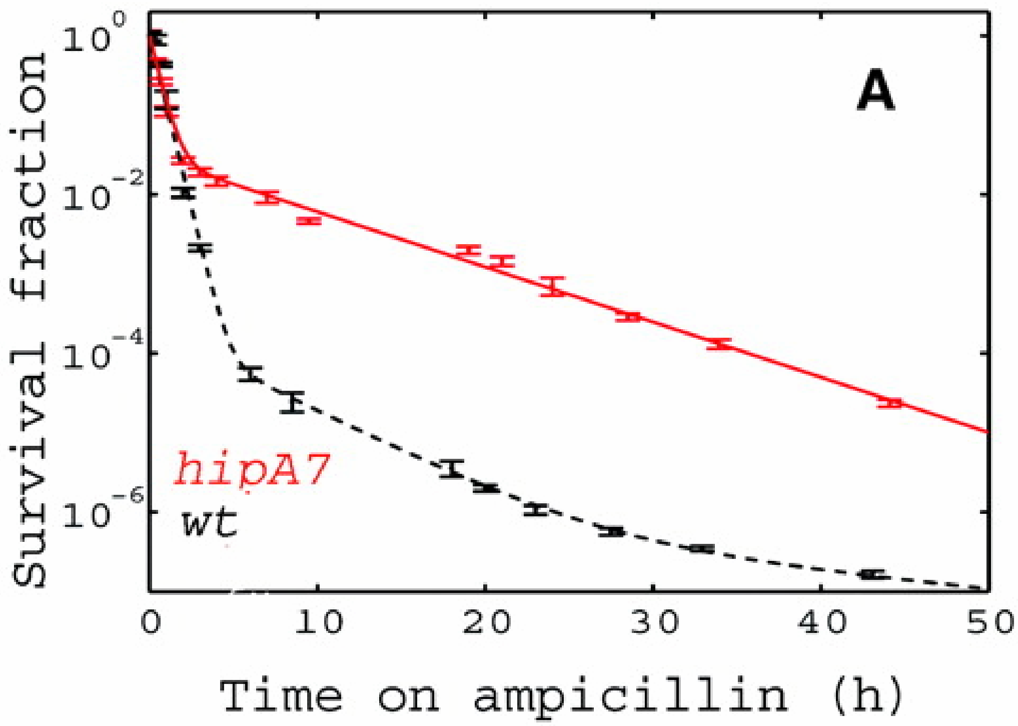
DARPA NLM

Penn
University of Pennsylvania

Bacterial persistence

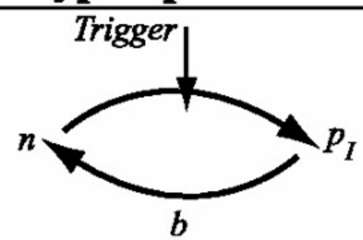
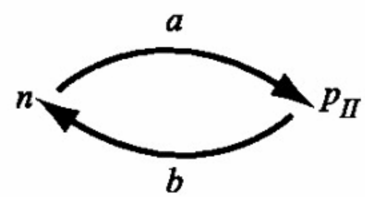
- Discovered as soon as antibiotics were used
- A fraction of an isogenic population survives antibiotic treatment significantly better than the rest

- If cultured, the surviving fraction gives rise to a population identical to the original one (...)
- Bimodal kill curves



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**
	
$\begin{cases} \frac{dp_I}{dt} = -bp_I + \mu_p p_I \\ \frac{dn}{dt} = bp_I + \mu_n n \end{cases}$ Eq.(1)	$\begin{cases} \frac{dn}{dt} = -an + bp_{II} + \mu_n n \\ \frac{dp_{II}}{dt} = an - bp_{II} + \mu_p p_{II} \end{cases}$ 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 were 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)

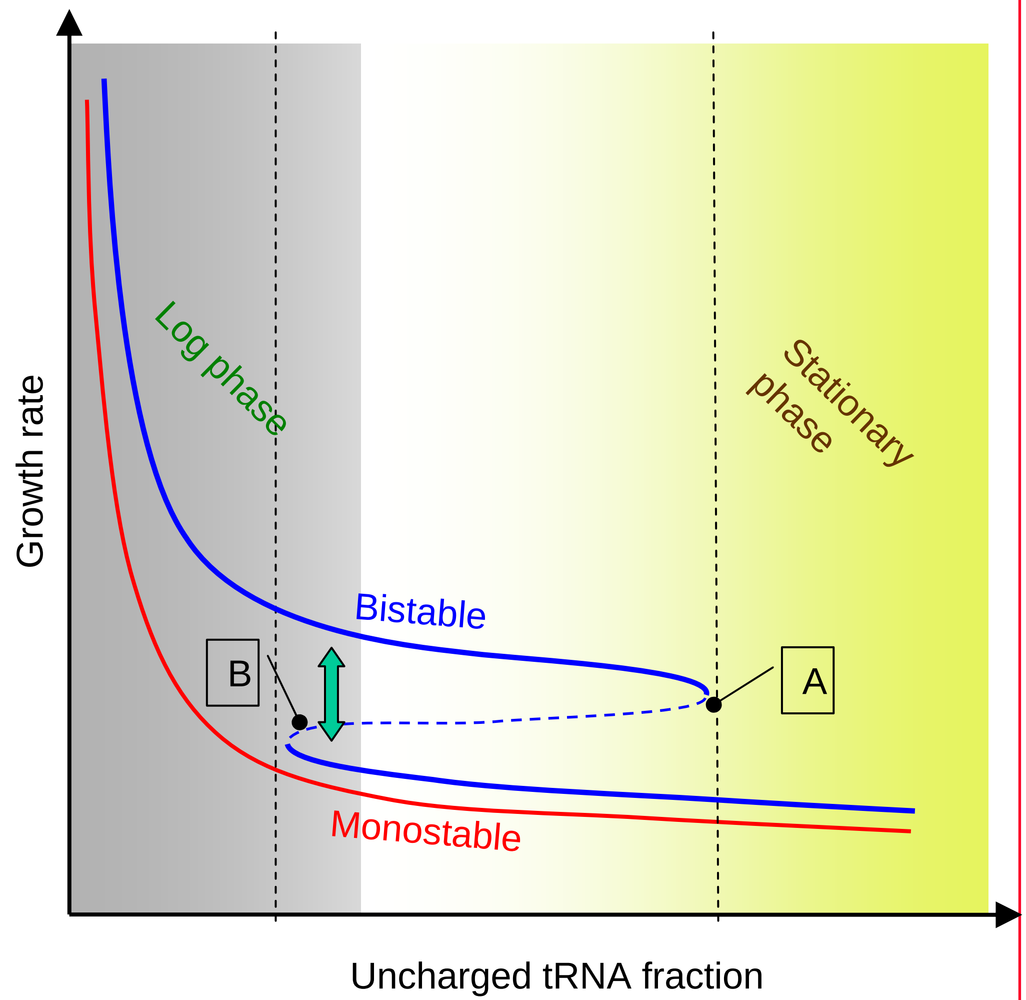
Persistence as an evolutionary advantage

- 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?

Stringent Response and *(p)ppGpp*

- Stringent response: induced by adverse environmental change:
  - Reduced nutrient (amino-acid) availability
  - Antibiotics
  - Many different stress stimuli

- 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*

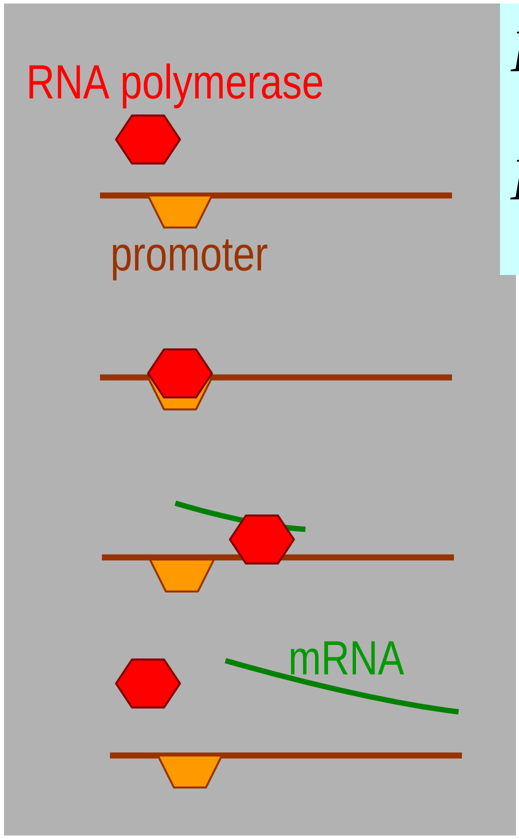
Mechanism of differential regulation

- 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 → less free RNAP
  - Gourse: RNAP affinity for stable promoters decreases → more free RNAP

Mechanism of differential regulation

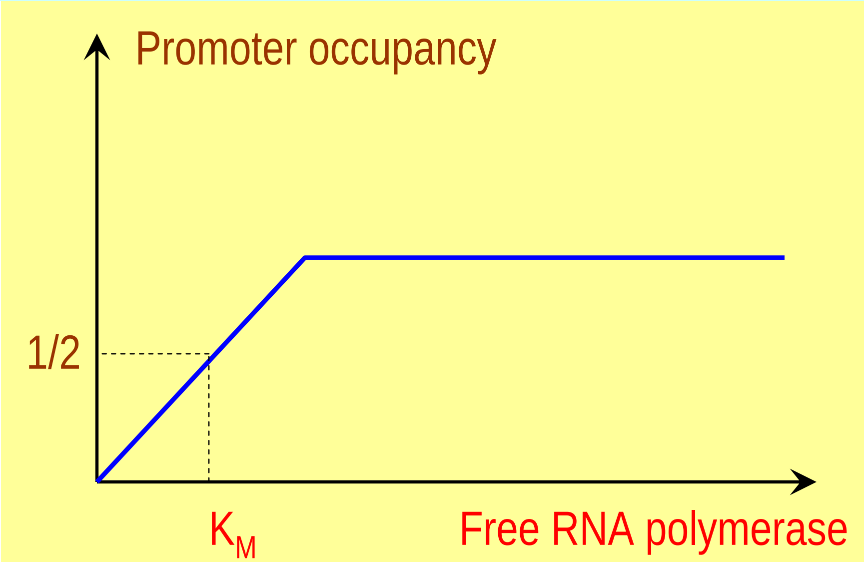
Describe transcription kinetics in terms of effective parameters

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



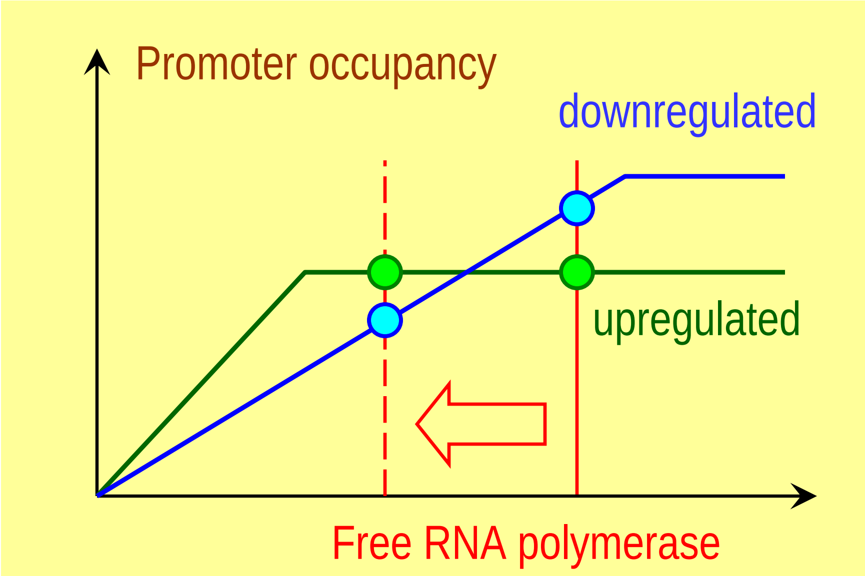
$$R + P \xleftrightarrow{k_1, k_2} RP \xrightarrow{k_3} R^* + P \xrightarrow{1/\tau} R + P + mRNA$$

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

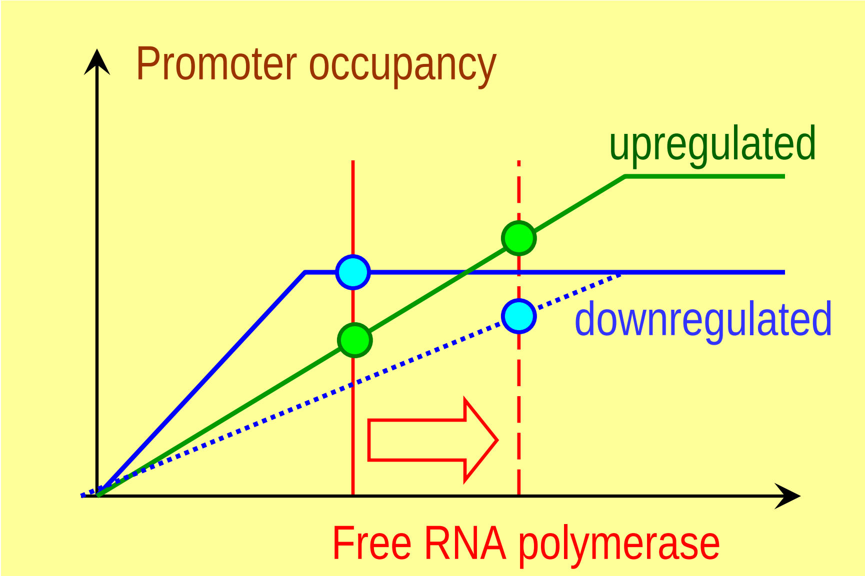


Mechanism of differential regulation

- Transcription time increases
- Free RNAP decreases



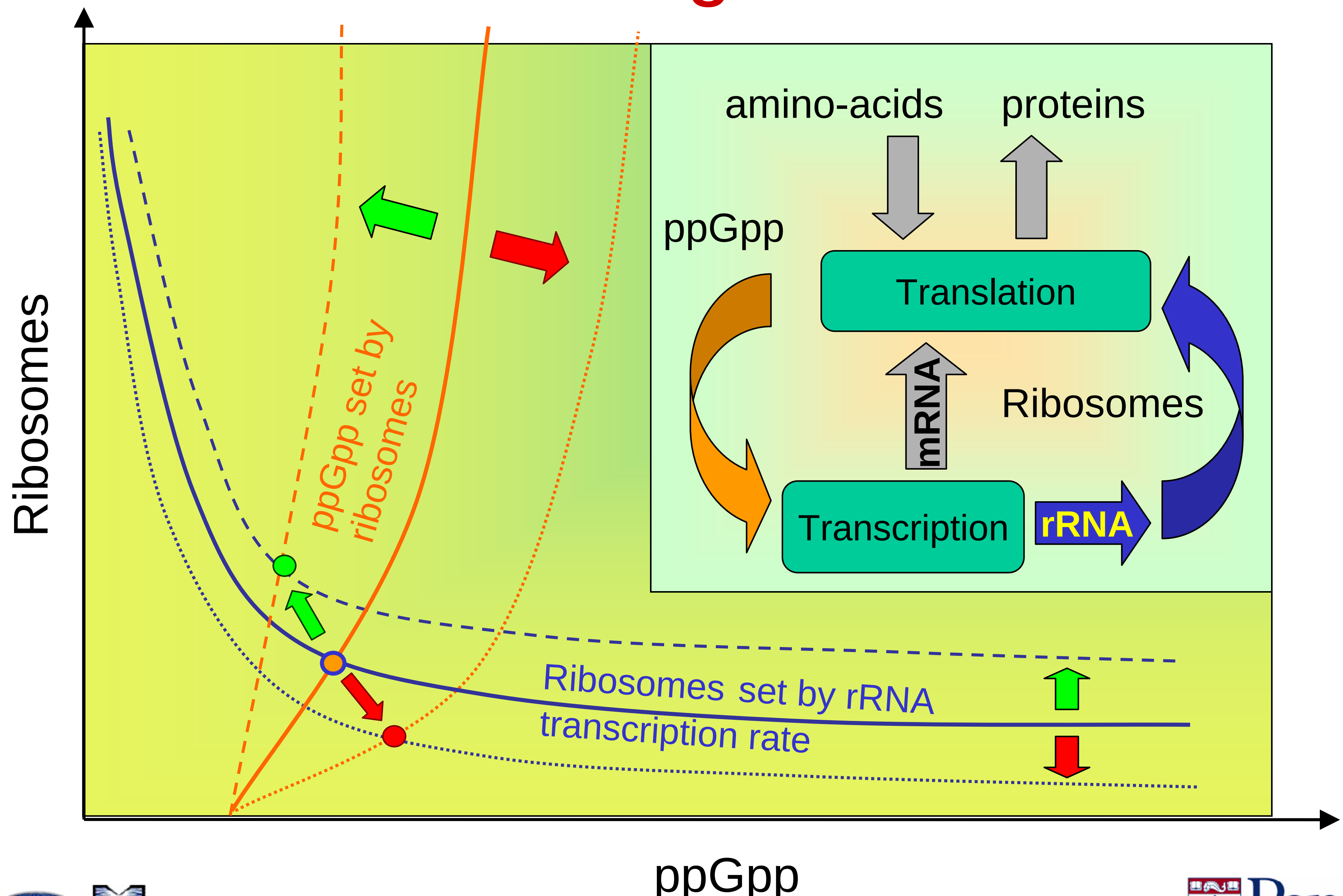
- k_2 of stable species increases
- Free RNAP increases



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 → (+) $tRNA^u$ →(+) RAC → (+) (p)ppGpp → (-) rRNA → (-) ribosomes → (-) RAC

Mechanism of growth control

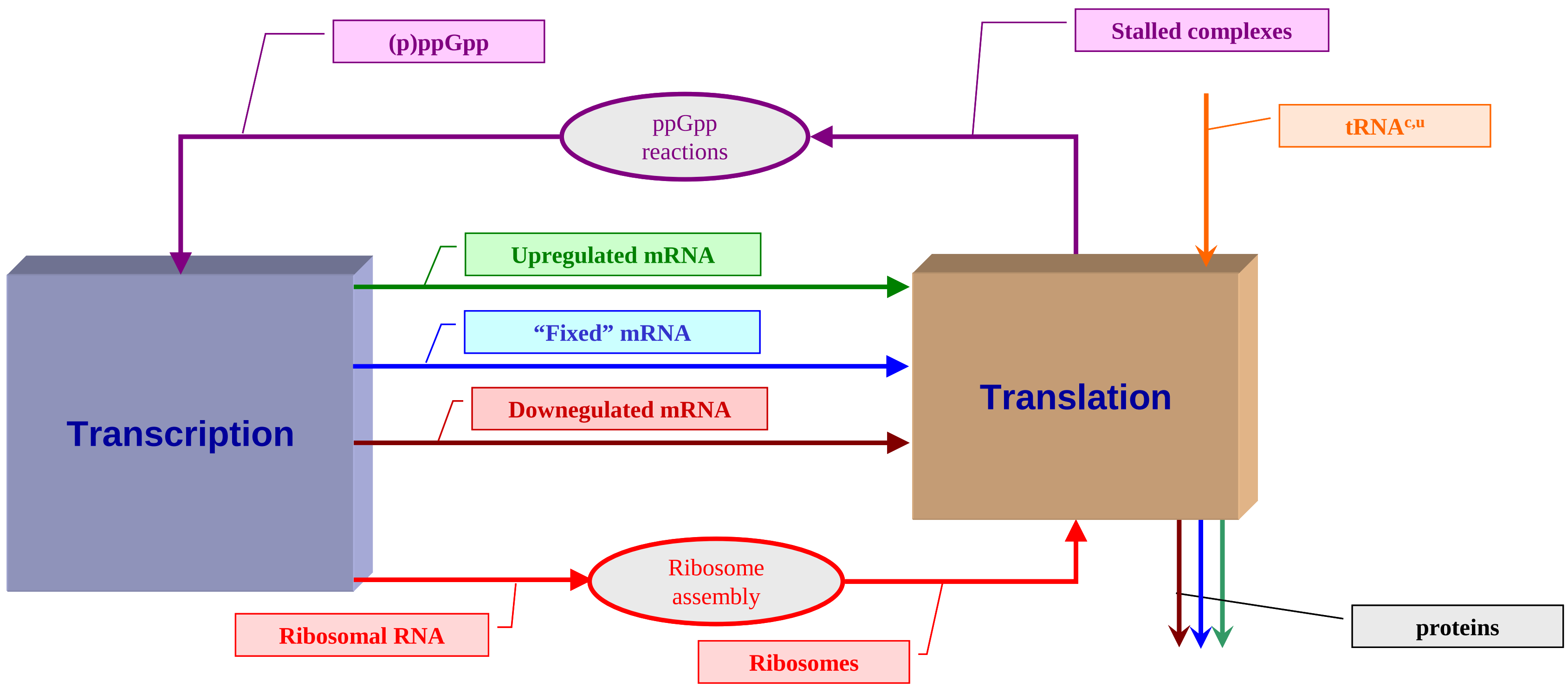


A wider role for *(p)ppGpp*

- 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



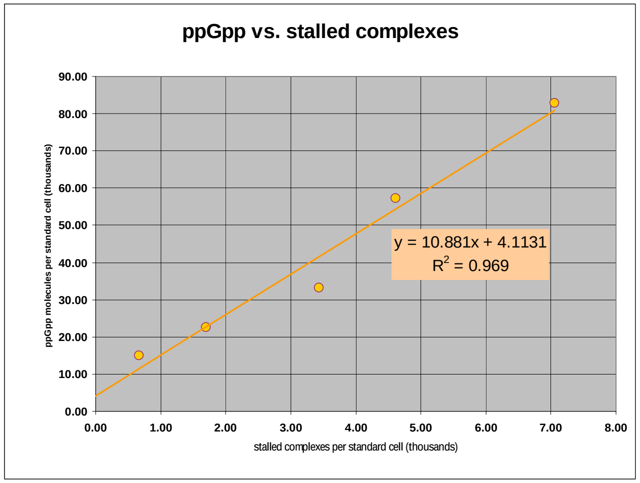
Parameter estimation

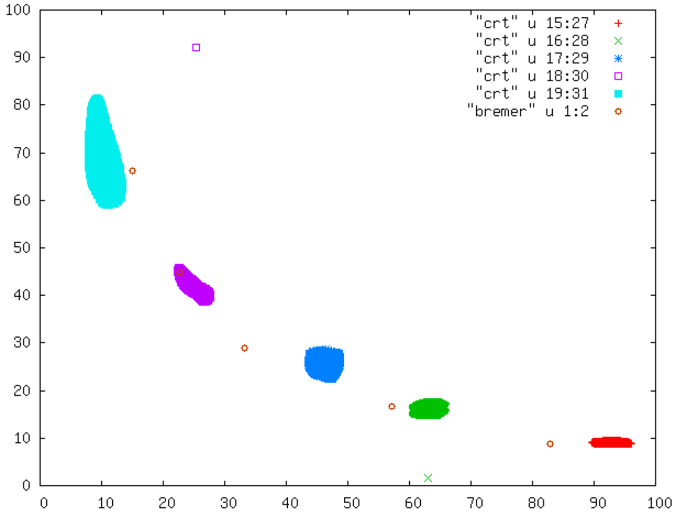
•Promoter counts in the different groups based on genomic data (Cheng 2002)

•Kinetic parameter values from:

- literature on stringent response
- direct estimation

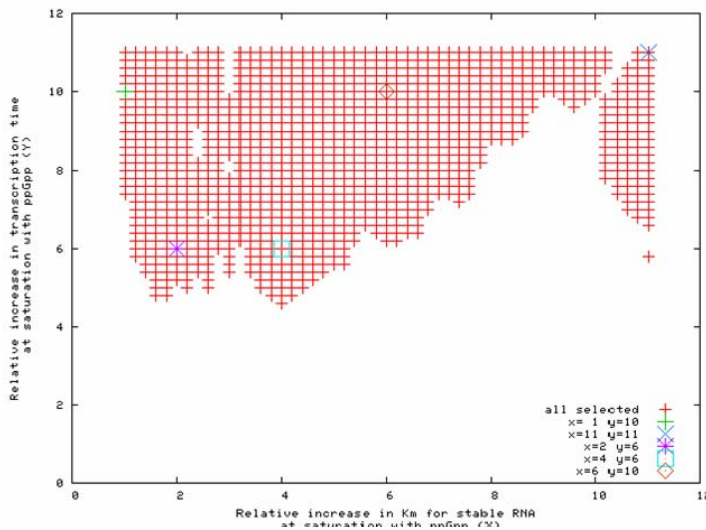
•A group of 8 parameters determined indirectly, by comparing model predictions of RNA transcription rates with the compilation of Bremer (1996)

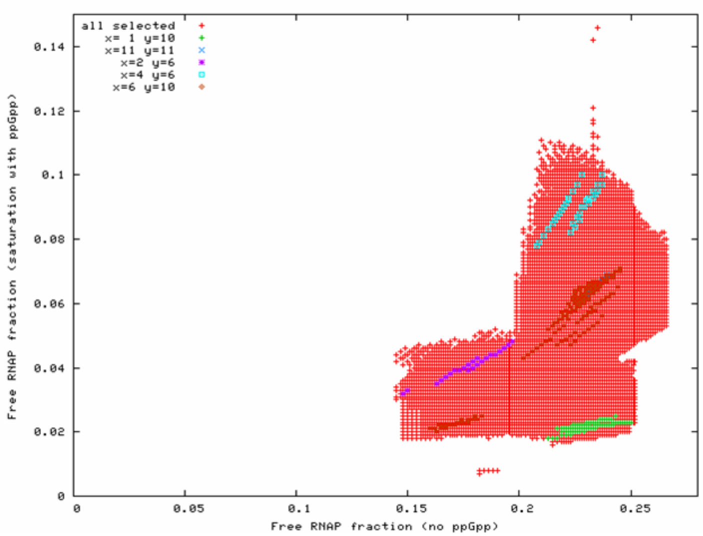




Parameter search

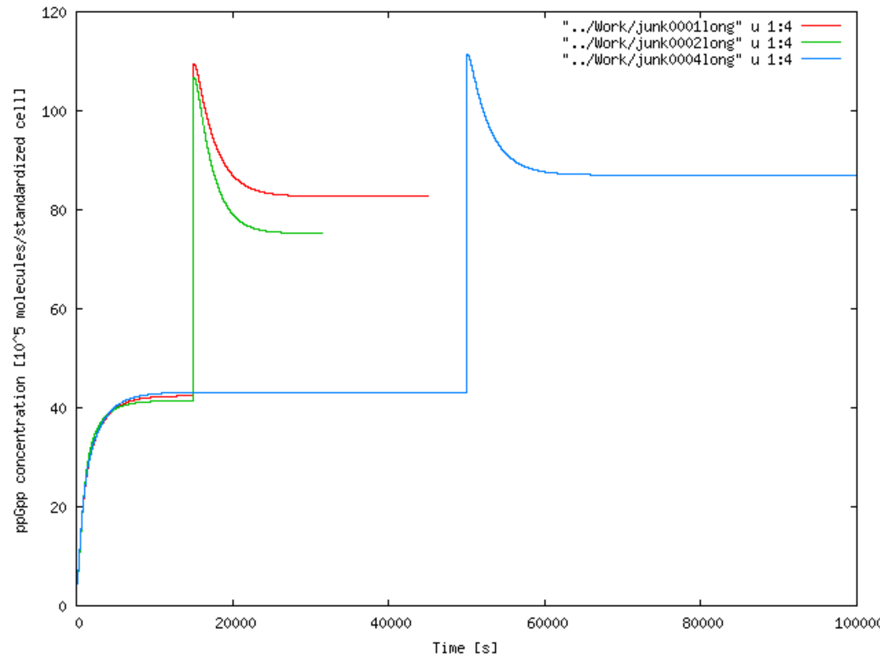
- Group of eight parameters
- Related to transcription kinetics
- Subject to dispute
- ‘Brute force’, sweep, using grid of 10^8 values
- Many acceptable sets found





Time dependent simulations

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



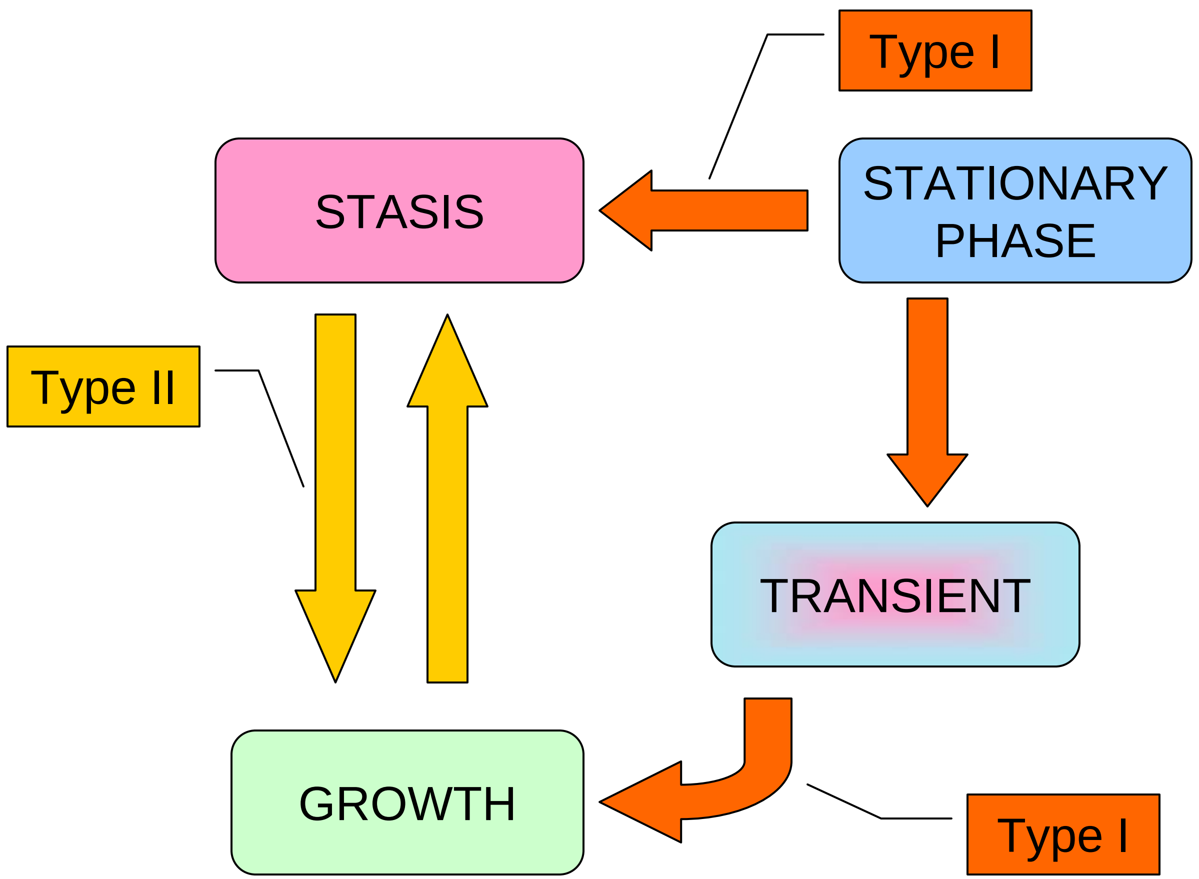
The 'other' stringent response: RelBE, tmRNA, lon

- 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

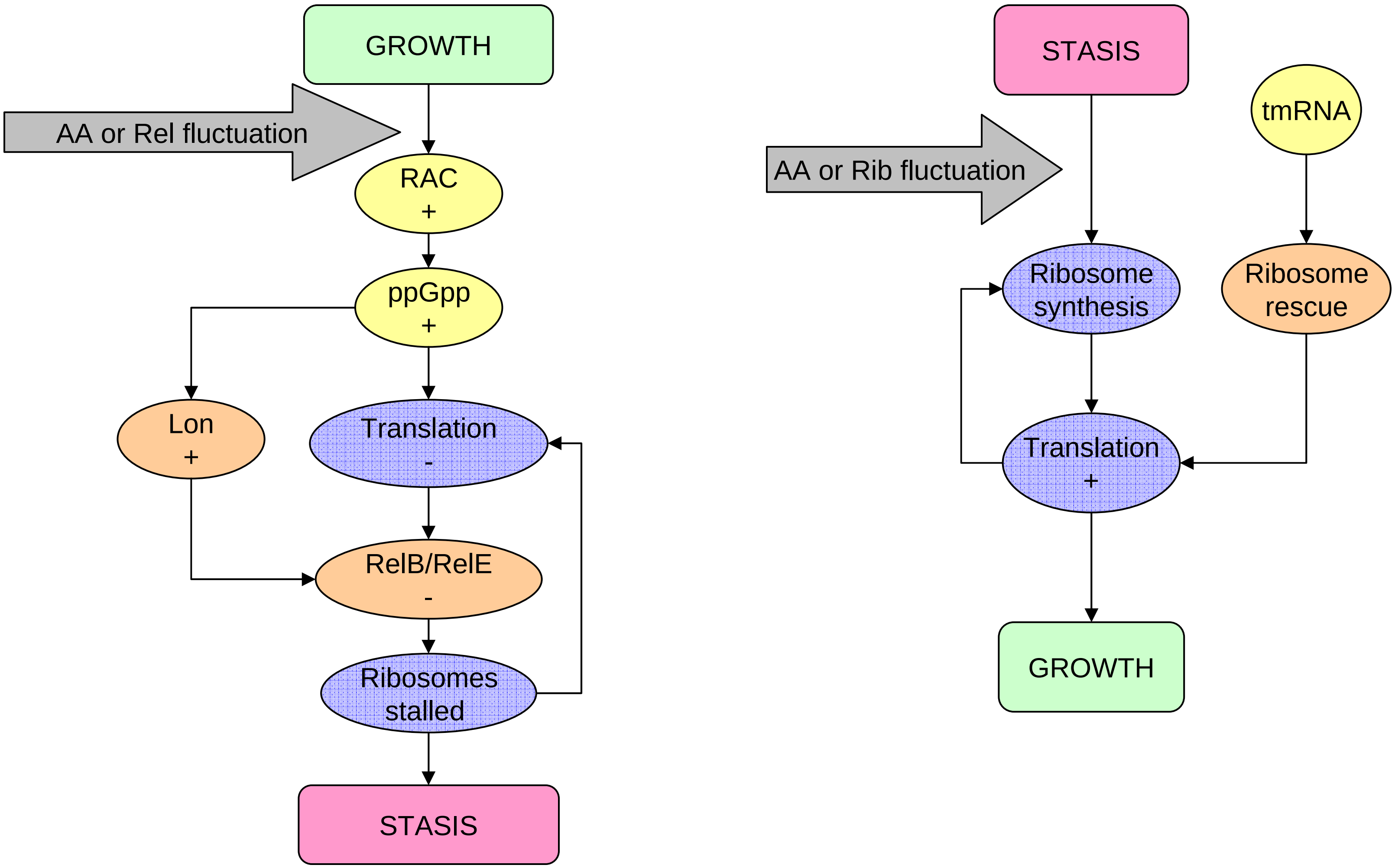
- Lon triggers a transient imbalance of RelB/RelE
  - Lon increases the degradation of RelE, creating an excess of RelB;
  - this leads to a drop of translation, so RelB eventually also degrades
  - At the end of this cycle, the cell has drastically reduced the number of *active* ribosomes → no more 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

Stochastic transitions facilitated by lon



Stochastic transitions facilitated by lon

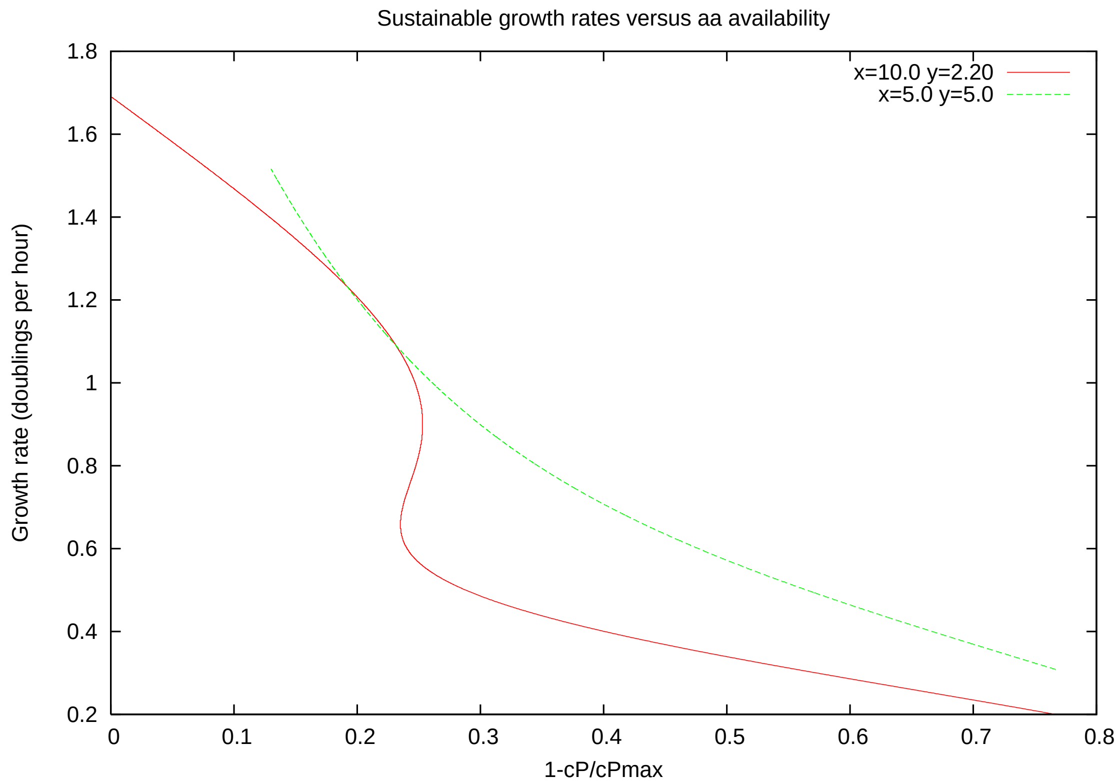


Bistability

- 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

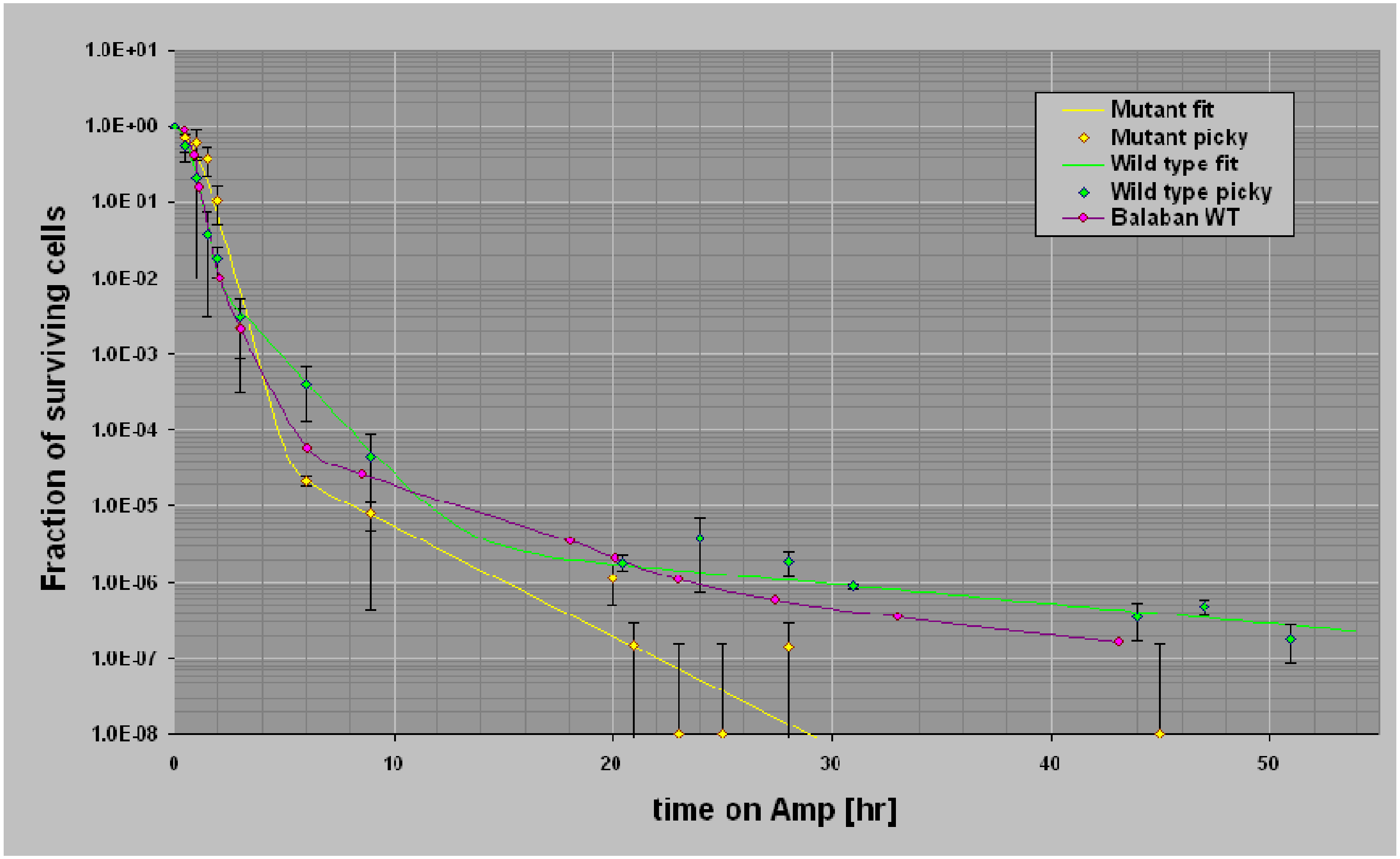
But, is bistability really necessary?

- If the lon 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



Experimental results

- Rel/spoT knockouts have severely diminished persistency phenotype:



Summary

- 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
- Mathematical model of growth rate control mechanism
  - Parameter sets chosen using both log phase and SR data
  - Introduction of RelBE/Lon necessary to ensure correct post-stringent behavior
- Model can accommodate bistability, but not for the best-match parameter sets
  - Exact mathematical bistability may not be necessary if Lon can be triggered by random fluctuations
- Experimentally, Rel/spoT knockouts lose persistence