A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler, Nature, 403, 2000

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Journal Club

Andreas Kühne

Introduction

- Networks of interacting biomolecules are important in living cells
- Network organization is poorly understood, despite intensive analysis of simple systems

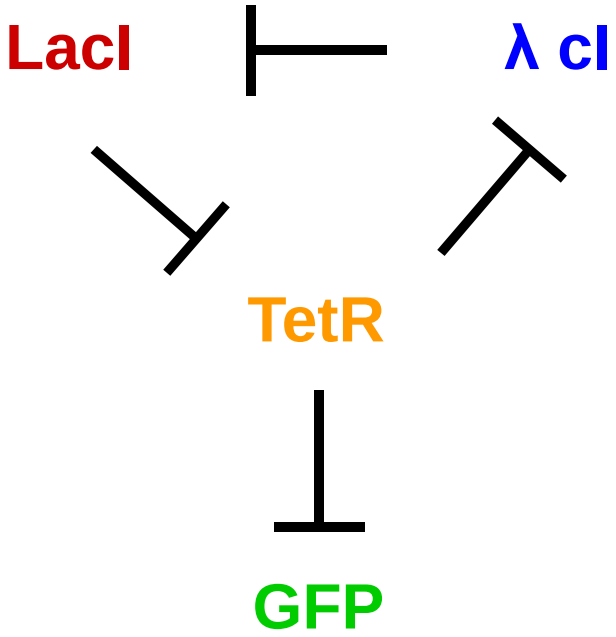
- Complementary Approach:

⇨ design and construction of a synthetic network with a certain function

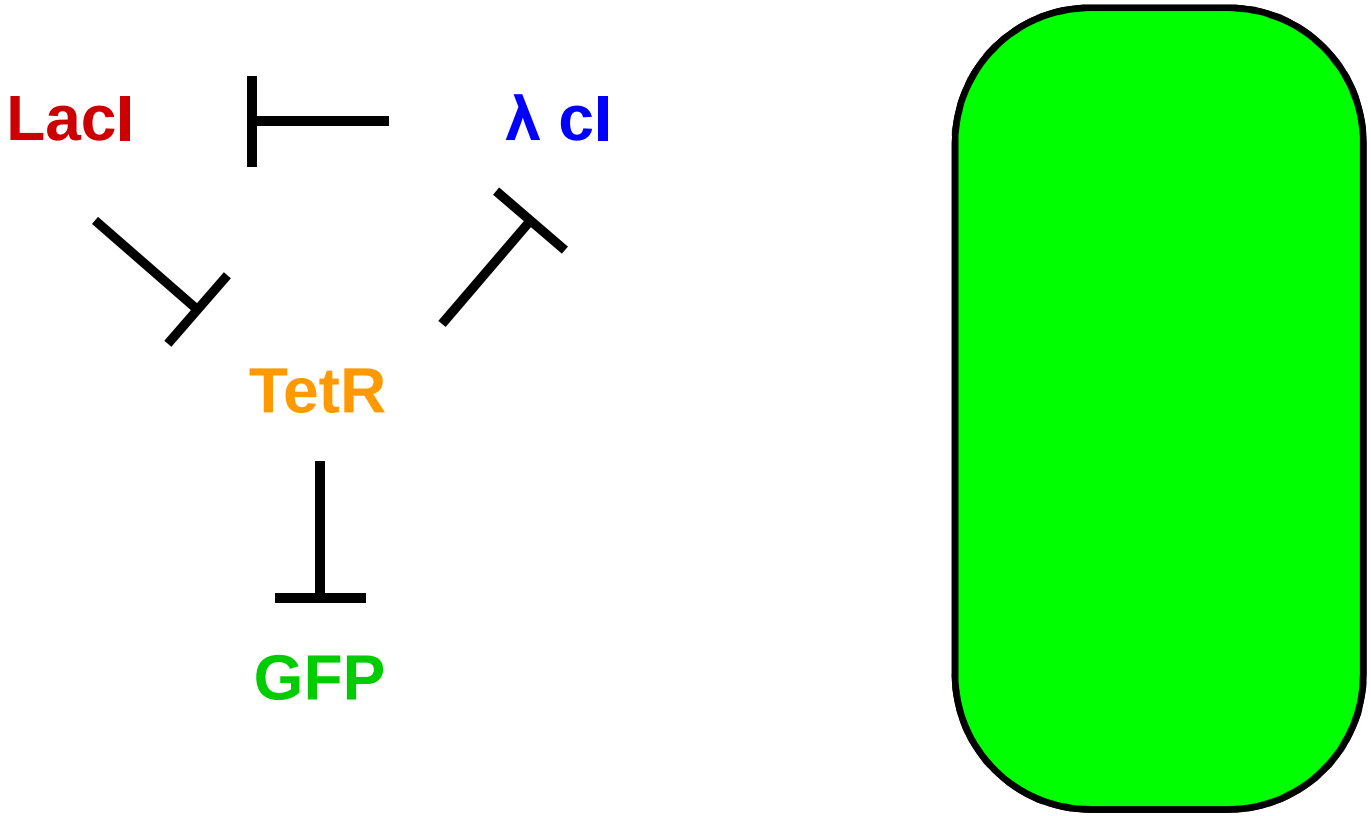
⇨ build an oscillating network in *E. coli* called “the repressilator”

The Repressilator: Design

- Negative feedbackloop of three transcriptional repressor systems, which are not part of a biological clock
  - LacI inhibits tetR expression
  - tetR inhibits cI expression
  - cI inhibits LacI expression
- Network induces synthesis of GFP
  - tetR inhibits GFP expression
- Resulting oscillations are slower then the cell-division cycle



The Repressilator: Design



Theoretical Model

- Simple mathematical model of transcriptional regulation
- System behavior could not be described exactly due to a lack of knowledge in molecular interactions inside the cell

⇨ Identify possible classes of dynamic behavior

⇨ Determine experimental parameters which have to be adjusted to obtain sustained oscillations

Theoretical Model

- Repressor protein concentrations p_i and mRNA concentrations m_i are continuous dynamical variables
- These dynamics depends on transcription, translation and degradation
- Repressors are treated identical except of their DNA-binding specifities

$$\frac{d\ m_i}{dt} = -m_i + \frac{\alpha}{\left(1 + p_j^n\right)} + \alpha_0$$

$$\frac{d\ p_i}{dt} = -\beta\left(p_i - m_i\right)$$

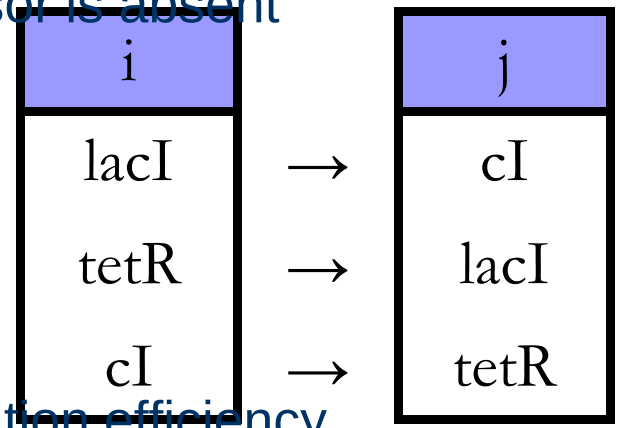
i		j
lacI	→	cI
tetR	→	lacI
cI	→	tetR

Theoretical Model

- α_0 protein copies per cell when promotor is saturated with repressor
- $\alpha+\alpha_0$ protein copies per cell when repressor is absent
- β ratio of protein and mRNA decay rate
- n Hill coefficient
- Timescale: mRNA lifetime
- Protein concentrations: units of K_M
- mRNA concentrations: rescaled by translation efficiency

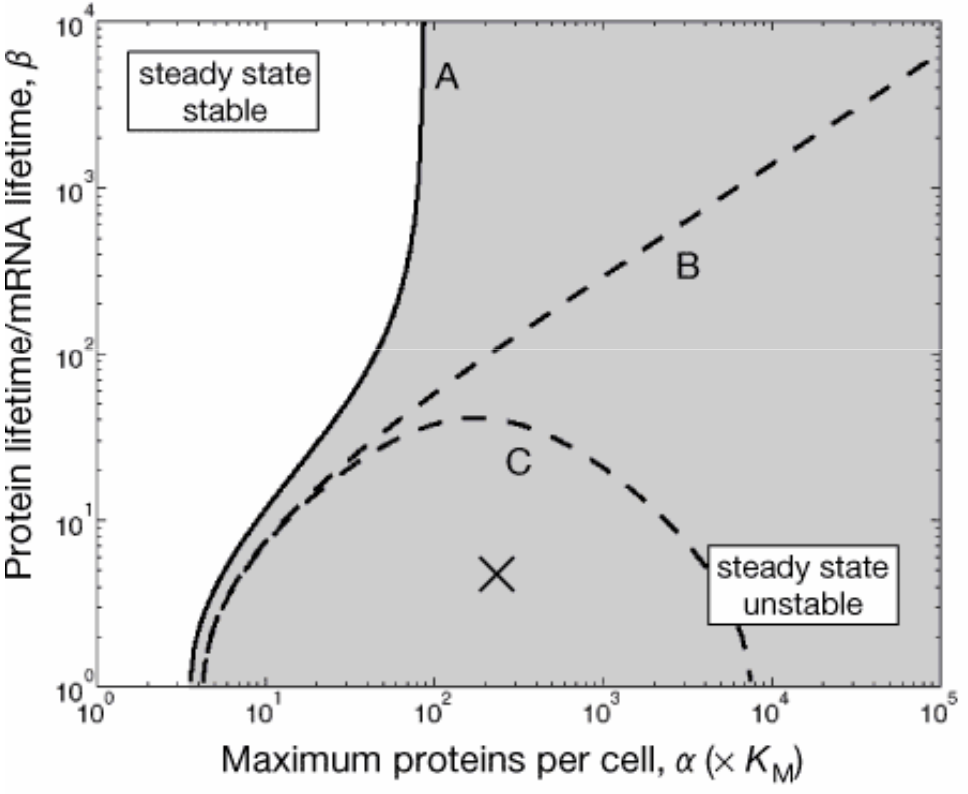
$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{\left(1+p_j^n\right)} + \alpha_0$$

$$\frac{dp_i}{dt} = \beta\left(p_i - m_i\right)$$



Theoretical Model: Stability Diagram

- Unique steady state solution: stable and unstable
- A, B, C boundary between stable and unstable region:
  - A: $n = 2.1$, $\alpha_0 = 0$
  - B: $n = 2.0$, $\alpha_0 = 0$
  - C: $n = 2.1$, $\alpha_0/\alpha = 10^{-3}$

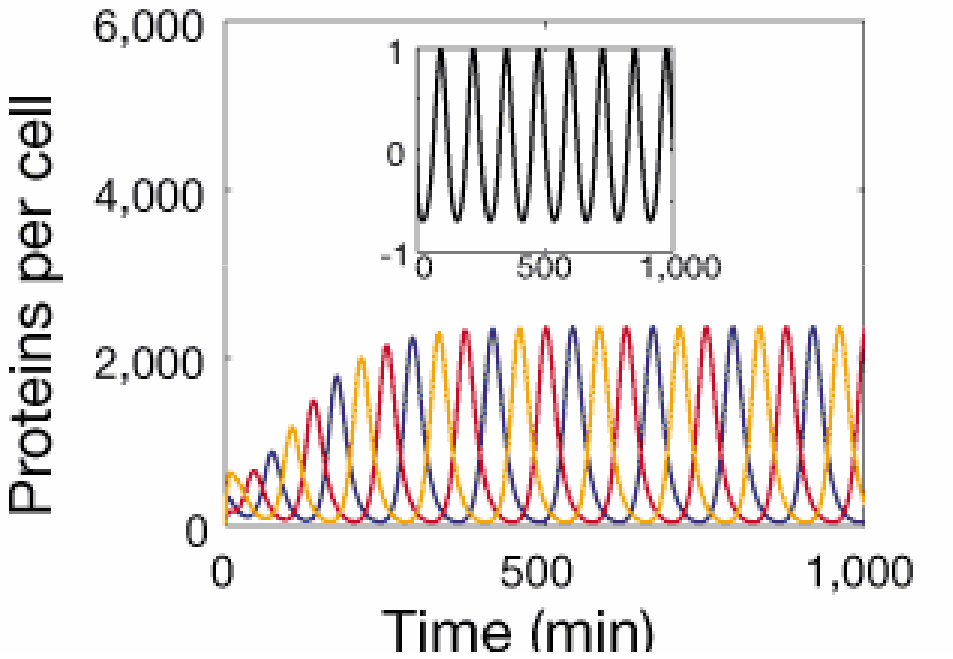


→ increasing Hill coefficients leads to no limitation of β for large α

→ if α_0 is comparable to K_M, the unstable domain shrinks

Theoretical Model: Numerical Solution

- Promoter strength: from 5 x 10^{-4} to 0.5 transcripts per second
- Average translation efficiency: 20 proteins per transcript
- Hill coefficient n = 2
- Protein half-life $T_{1/2,p}$ = 10 min, mRNA half-life $T_{1/2,mRNA}$ = 2 min
- K_M = 40 monomers per cell



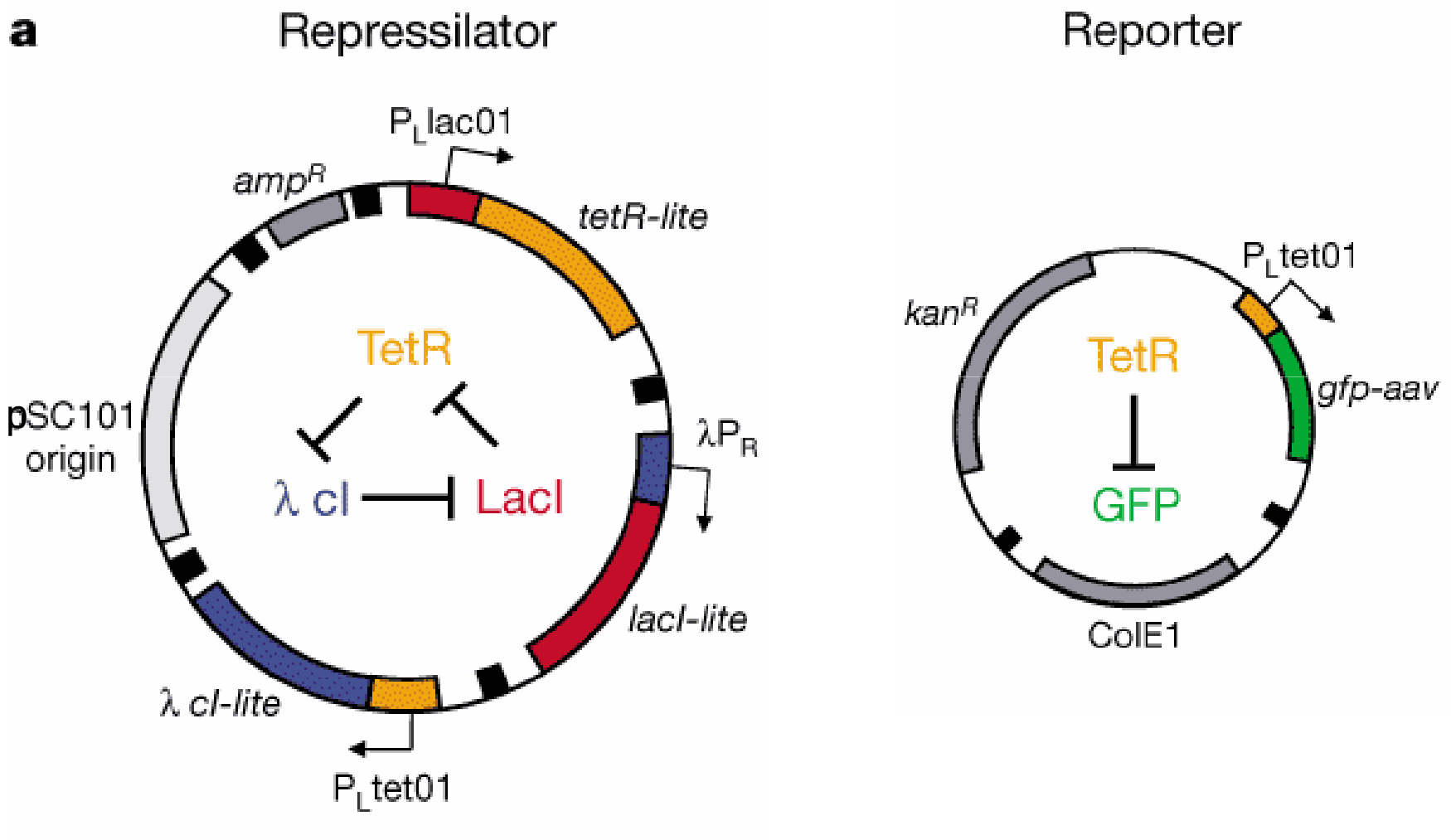
Experimental Setup

- Mathematical Model shows that negative feedback loops can lead to oscillations in protein concentrations
- Oscillations are favored by:
  - Strong promoters coupled to efficient RBS
  - Tight transcriptional repression (low “leakiness”)
  - Cooperative repression characteristics
  - Comparable protein and mRNA decay rates
- Alterations of natural components
  1. Combine strong, tightly repressible λ P_L hybrid promoter with lac and tet operator sequence
  2. Reduce protein lifetime of repressor proteins with carboxy-terminal tags (ssrA RNA)

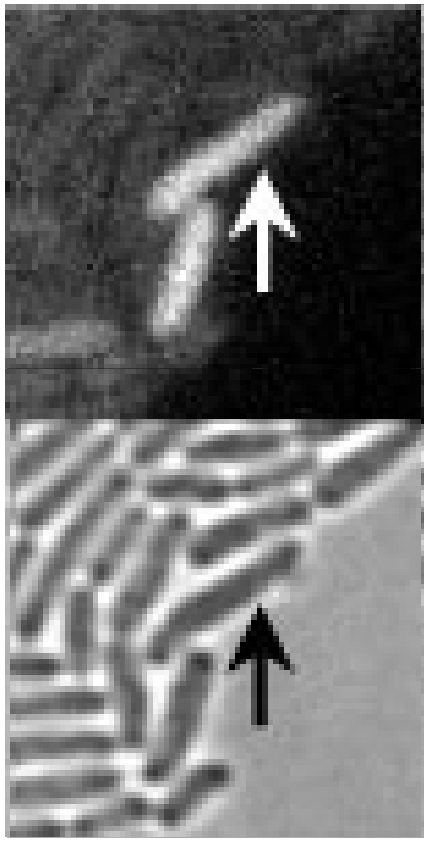
Experimental Setup: ssrA RNA

- *ssrA* gene codes for the *10Sa RNA* which codes for an 11 aa peptid residue
- This residue is linked to the C-terminal side of truncated protein products during translation
- Proteases, e.g. *Tsp*, recognize this tag and degrade the protein
- Fusion proteins with the ssrA gene are degraded faster: $T_{\lambda\text{-Repressor}}$ = 4 min instead of 60 min

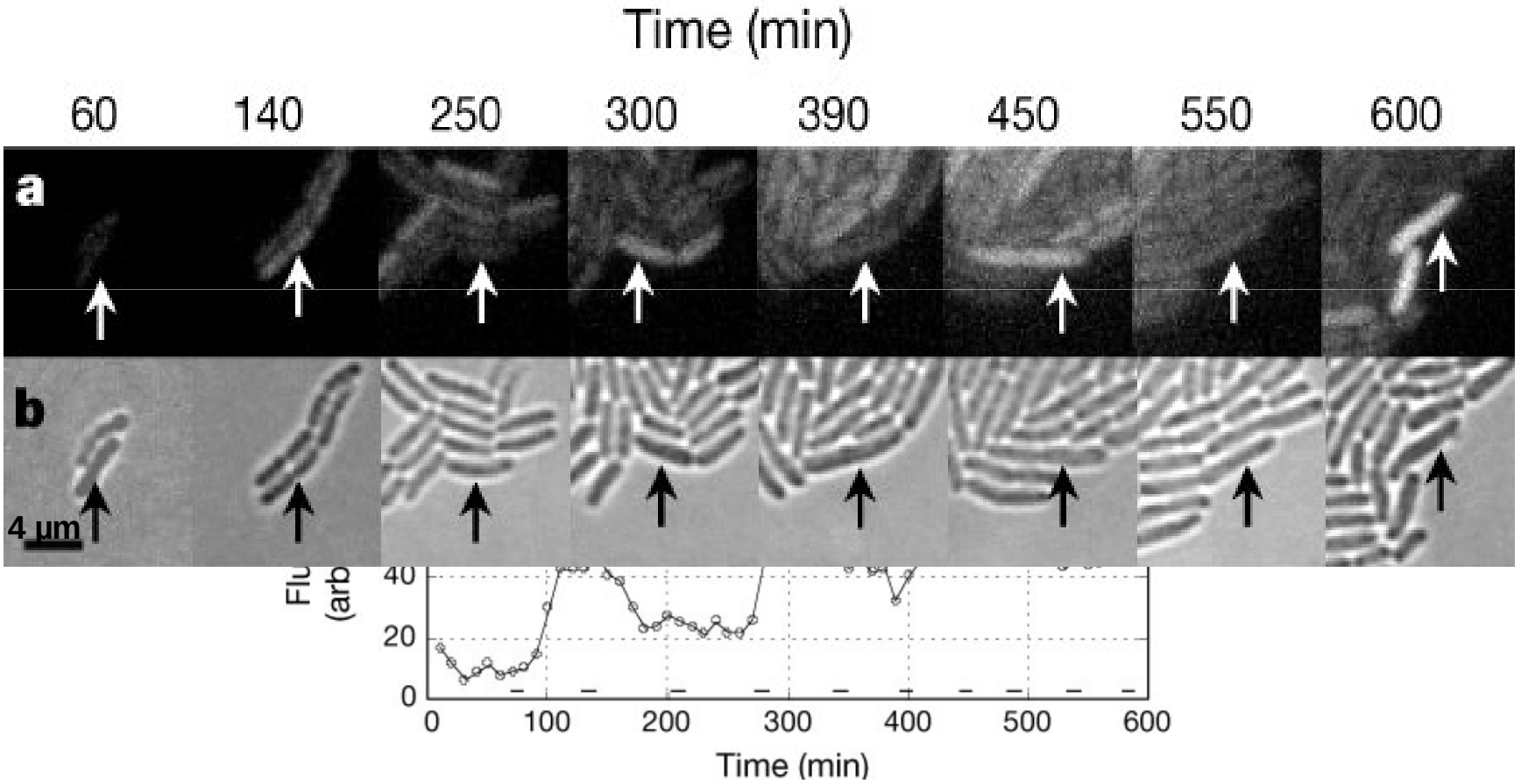
The Repressilator: Plasmid Design



Experimental Results



Experimental Results



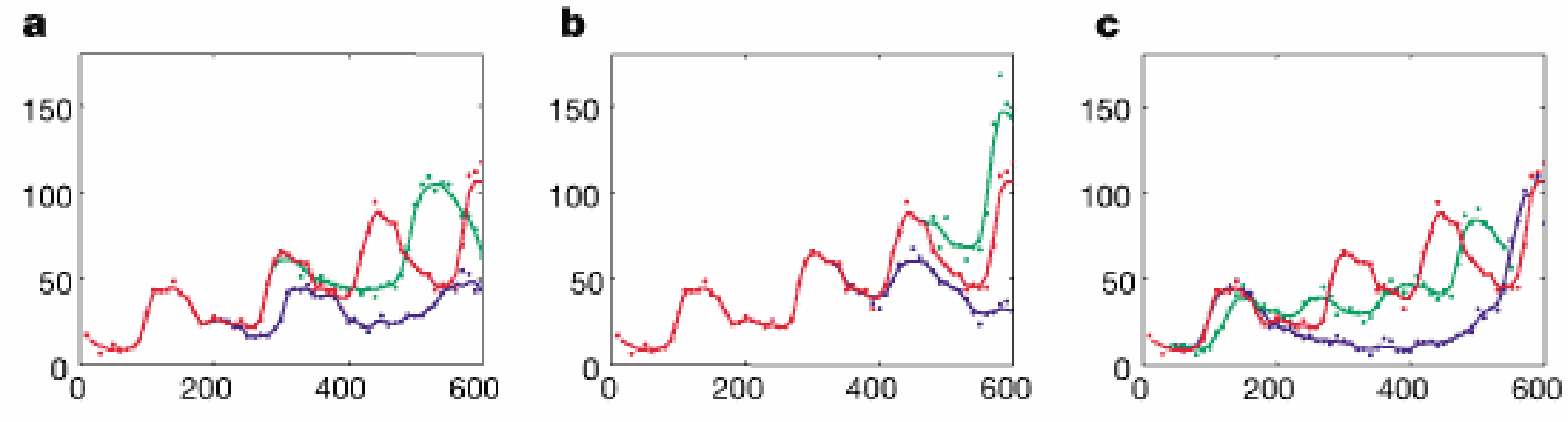
Experimental Results

- Fluorescence intensity of 100 individual tracked cell lineages was quantified (30 ℃)
- 40% exhibit oscillatory behaviour
- Mean periods: T = 160 +/- 40 min (3 x cell-division time)

Experimental Results: Synchronization

- IPTG interferes with repression by LacI
- A short pulse of IPTG could be able to synchronize a repressilator-population
- *E. coli* population grew over night in media containing IPTG, showed no oscillation
- After transfer to media lacking IPTG, they showed a single damped oscillation

Experimental Results

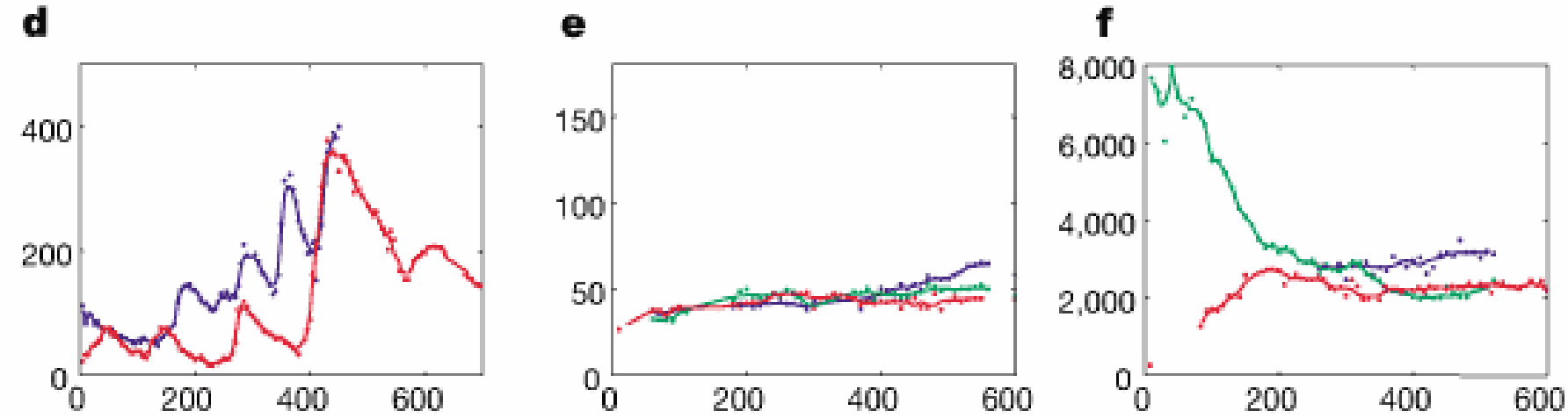


- GFP levels of sibling cells remain correlated for 95 +/- 10 min after septation (typical cell division time 50-70 min)

  ⇒ network state is transmitted to progenity cell

- Observed effects:
  - Post septation phase delays (a)
  - Phase maintained, amplitude varies significantly (b)
  - Reduced period (green) and long delay (blue) (c)

Experimental Results



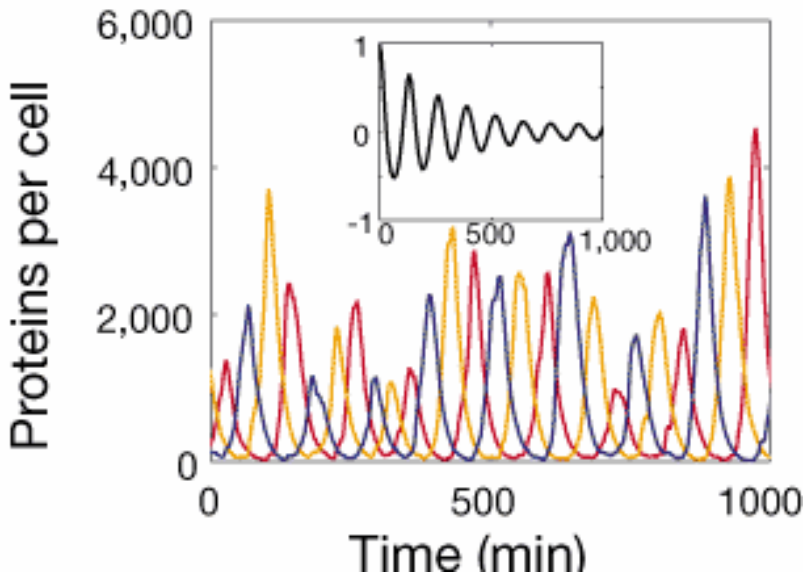
- Variability in different experiments (d): large variability in amplitude and period of oscillations

- Negative Controls (e, f)

  (e) Oscillation was inhibited by 50 µM IPTG in the media
  (f) Cells only with reporter plasmids

Experimental Results: Noise

- Results show strong influence of noise
- Recent work (McAdams *et al.*, 1999) has shown that stochastic effects may be responsible for noise in gene-expression networks
- Stochastic simulations shows large variability



Comparison With Circadian Clocks

- Circadian rhythms in Cyanobacteria: Oscillating system which have a longer period then cell division time
- Reliable oscillation in contrast to the noisy and variable one of the repressilator
- Circadian oscillators use positive and negative control elements
- Barkai et al. have shown in theoretical analysis that combination of positive and negative elements lead to bistable behavior and high noise-resistance behavior
- Further design of an oscillating network consisting of positive and negative control elements can possibly give an conclusion for noise and temperature resistance

Summary

- Design and construction of an artificial genetic network with new functional properties
- Use of parts which come from other contexts in nature
- Work is analog to the design of functional proteins out of different motifs
- Further characterization of components and alteration of network connectivity provide a basis for the design of applications
- Network design can help to understand design principles of natural genetic networks

Thanks for your attention!

References

(1) **Michael B. Elowitz & Stanislas Leibler,** *A synthetic oscillatory network of transcriptional regulators,* Nature, 403, 2000

(2) **Andersen, J. B. et al.** *New unstable variants of green Fluorescent protein for studies of transient gene xpression in bacteria*. Appl. Environ. Microbiol. 64, 2240±2246 (1998)

(3) **Keiler, K. C., Waller, P. R. & Sauer, R. T.** *Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA.* Science 271, 990±993 (1996).