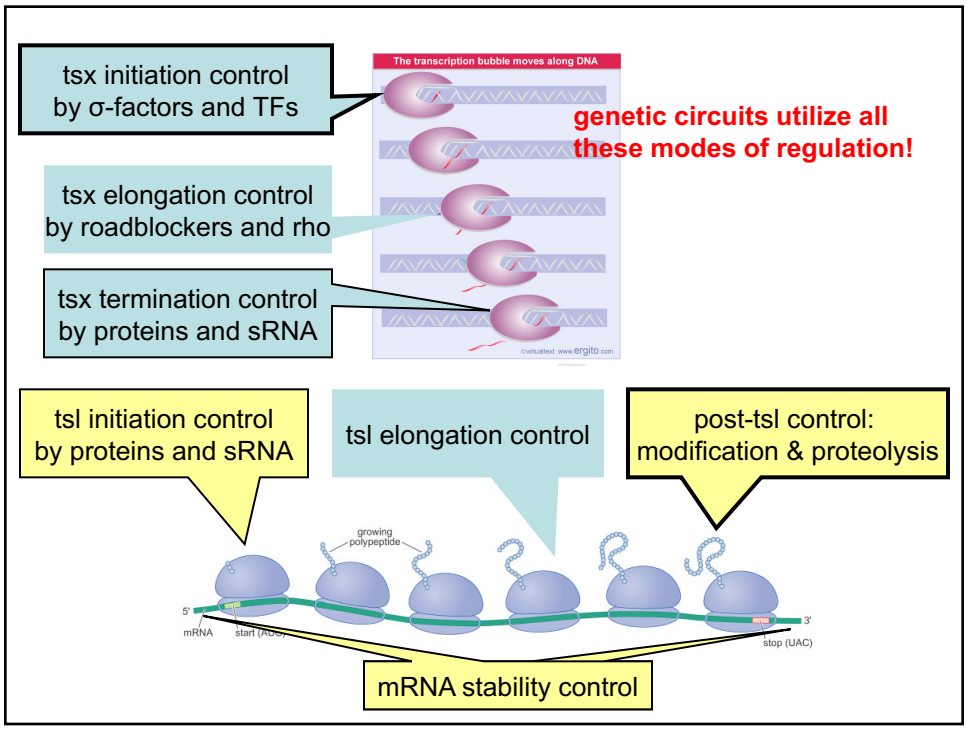



1

# Topic 4: Genetic Circuits

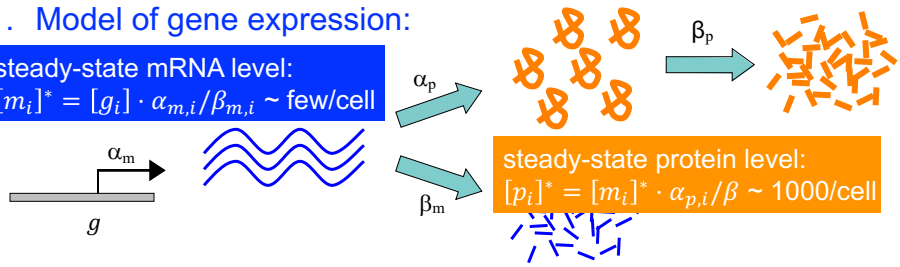
A. **Models and behaviors of simple genetic circuits**
   1. general model of gene expression
   2. negative autoregulation
   3. positive auto-regulation
   4. oscillators
B. **Noise in gene expression**
C. **Metabolic control**

2

# A. Models and behaviors of simple genetic circuits

## 1. Model of gene expression:

steady-state mRNA level:
$[m_i]^* = [g_i] \cdot \alpha_{m,i}/\beta_{m,i}$ ~ few/cell




steady-state protein level:
$[p_i]^* = [m_i]^* \cdot \alpha_{p,i}/\beta$ ~ 1000/cell

$g$ = # promoters per cell; 1-8/cell for chromosomal promoters; can be >100 on plasmids
$\alpha_m = \alpha_{m,0}\,\mathcal{P}$ mRNA synthesis rate per promoter; max ~ 1/sec; typical ~ 1/min
$\beta_m^{-1}$ = mRNA life time; typical 1~2 min; max ~ doubling time
$\alpha_p$ = protein synthesis rate per mRNA; typical high ~ 10/min
$\beta_p^{-1}$ = protein life-time; max ~ infinite; short ~ min

} 10~20 proteins/mRNA ("burstiness")

mean-field description (rate eqn) for gene $i$
$[g_i]$ = conc of promoters
$[m_i]$ = conc of functional mRNA transcript
$[p_i]$ = conc of protein product

$$\begin{cases} \frac{d}{dt}[m_i] = \alpha_{m,i}[g_i] - \beta_{m,i}[m_i] \\ \frac{d}{dt}[p_i] = \alpha_{p,i}[m_i] - \underbrace{(\beta_{p,i} + \lambda)}_{\beta}[p_i] \end{cases}$$

growth rate

3

**not contained** in the simple model:

- delay from tsx init to completion of mRNA synthesis
  [typically ~ 0.5 min, could be longer with roadblocks, tsx pauses, etc]
- delay in protein synthesis (~ min) and/or maturation (can be > 10 min)
- fluctuations within one cell-doubling
  - dilution due to (exponential) growth of cell volume $V_{cell}(t)$
  - gene dose change (differential increase of $g(t)$ and $V_{cell}(t)$ during cell cycle)
  - changes in conc of RNAp (➔$\alpha_m$), ribosomes (➔$\alpha_p$), RNases (➔$\beta_m$), proteases (➔$\beta_p$), global regulators, etc.
- stochasticity in transcription, translation, degradation, and cell division
- stochastic variations in cellular growth rate

➔ simple rate eqn (+ stochastic improvement) not faithfully applicable to time scales ≲ cell doubling time
[very short-time dynamics okay; e.g., allosteric control, protein modifications]

➔ for time scales ≫ cell doubling time, parameters are well-defined in **exponential growth phase**

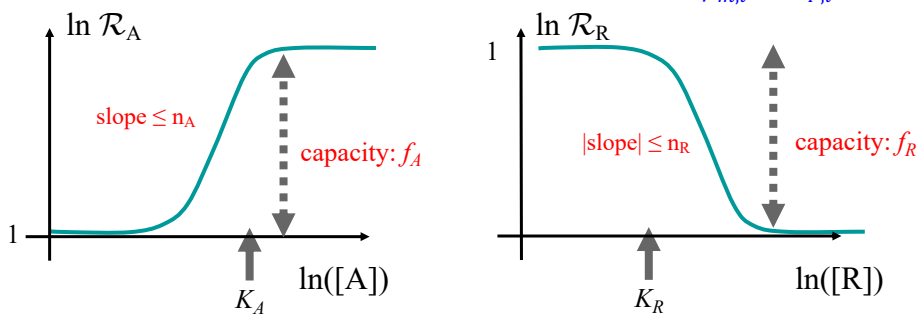
set $d[\mathrm{m}]/dt = 0$ (fast) to get $\frac{d}{dt}[p_i] = \alpha_i[g_i] - \beta_i[p_i]$ where $\alpha_i = \alpha_{m,i}\alpha_{p,i}/\beta_{m,i}$
[note: $\alpha_{p,i}/\beta_{m,i}$ ~ # transcripts per mRNA life-time = "burstiness" of mRNA $i$ ]

➔ all parameters **growth-rate dependent** (next major topic); fixed for now.

4

Below, we will explore behaviors of simple genetic circuit motifs using mechanisms of tsx control; will illustrate the effect of post-transcriptional control which enhances cooperativity

consider tsx init control only (for simplicity),
with $[m_i^*] = \alpha_{m,0}\mathcal{P}_i/\beta_{m,i} = m_{0,i} \cdot \mathcal{R}$ $\quad m_{0,i} = \frac{\alpha_{m,0}}{\beta_{m,i}} \frac{[RNAP]_{av}}{K_{P,i}}$




$$\mathcal{R}_A = \frac{1 + f_A \left(\frac{[A]}{K_A}\right)^{n_A}}{1 + \left(\frac{[A]}{K_A}\right)^{n_A}} \qquad \mathcal{R}_R = \frac{1 + f_R^{-1} \left(\frac{[R]}{K_R}\right)^{n_R}}{1 + \left(\frac{[R]}{K_R}\right)^{n_R}}$$

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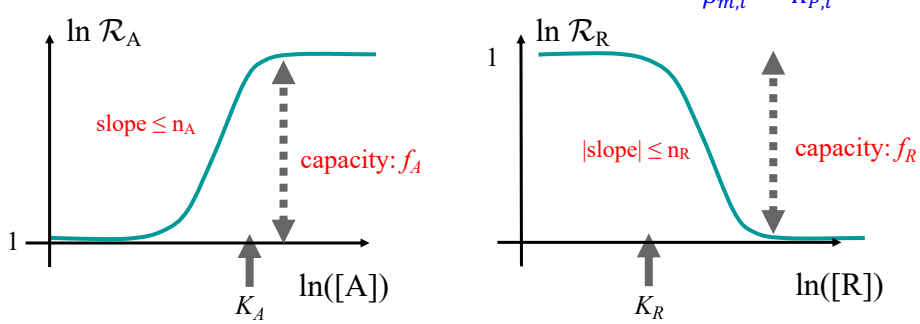
in terms of protein dynamics $\frac{d}{dt}[p_i] = \alpha_i - \beta_i \cdot [p_i]$

$$\alpha_i = \alpha_{p,i} \cdot [m_i^*] = \alpha_{p,i} m_{0,i} \cdot \mathcal{R}_i \equiv \alpha_{0,i} \cdot \mathcal{G}_i$$

$$\beta_i = \beta_{p,i} + \lambda$$

max rate: $\mathcal{G}_i = \mathcal{R}_i / \max(\mathcal{R}_i)$

consider tsx init control only (for simplicity),
with $[m_i^*] = \alpha_{m,0}\mathcal{P}_i/\beta_{m,i} = m_{0,i} \cdot \mathcal{R}$ $\quad m_{0,i} = \frac{\alpha_{m,0}}{\beta_{m,i}} \frac{[RNAP]_{av}}{K_{P,i}}$




$$\mathcal{R}_A = \frac{1 + f_A \left(\frac{[A]}{K_A}\right)^{n_A}}{1 + \left(\frac{[A]}{K_A}\right)^{n_A}} \qquad \mathcal{R}_R = \frac{1 + f_R^{-1} \left(\frac{[R]}{K_R}\right)^{n_R}}{1 + \left(\frac{[R]}{K_R}\right)^{n_R}}$$

9

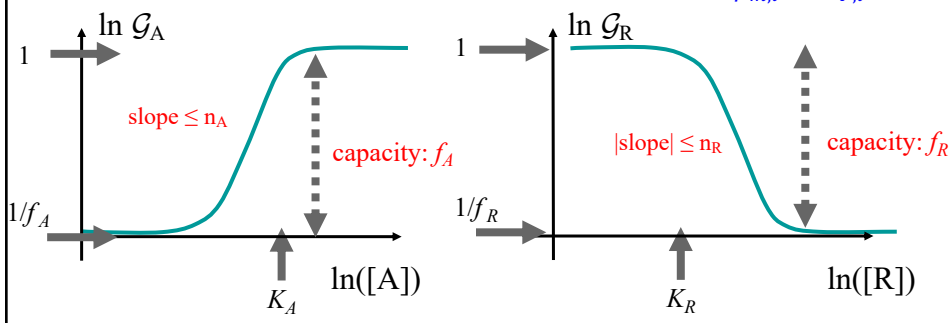
in terms of protein dynamics $\frac{d}{dt}[p_i] = \alpha_i - \beta_i \cdot [p_i]$

$$\alpha_i = \alpha_{p,i} \cdot [m_i^*] = \alpha_{p,i} m_{0,i} \cdot \mathcal{R}_i \equiv \alpha_{0,i} \cdot \mathcal{G}_i$$

$$\beta_i = \beta_{p,i} + \lambda$$

max rate: $\mathcal{G}_i = \mathcal{R}_i / \max(\mathcal{R}_i)$

consider tsx init control only (for simplicity),
with $[m_i^*] = \alpha_{m,0}\mathcal{P}_i/\beta_{m,i} = m_{0,i} \cdot \mathcal{R}$ $\quad m_{0,i} = \frac{\alpha_{m,0}}{\beta_{m,i}} \frac{[RNAP]_{av}}{K_{P,i}}$




$$\mathcal{G}_A = \mathcal{R}_A/f_A = \frac{f_A^{-1} + \left(\frac{[A]}{K_A}\right)^{n_A}}{1 + \left(\frac{[A]}{K_A}\right)^{n_A}} \qquad \mathcal{G}_R = \mathcal{R}_R = \frac{1 + f_R^{-1}\left(\frac{[R]}{K_R}\right)^{n_R}}{1 + \left(\frac{[R]}{K_R}\right)^{n_R}}$$

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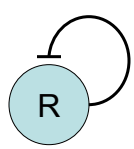
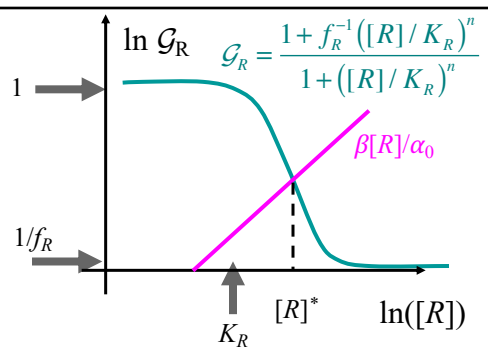
## 2. Negative autoregulation

(a very common network motif)




$$\frac{d}{dt}[R] = \alpha_0 \mathcal{G}_R([R]/K_R) - \beta[R]$$




assume circuit 'properly' biased: $K_R > [R]^* > f_R^{1/n} K_R$ or $K_R < \alpha_0/\beta < K_R f_R^{1/n}$

steady stat soln: $\frac{[R]^*}{K_R} = \left(\frac{\alpha_0}{\beta K_R}\right)^{1/(n+1)}$

➔ general dependence of parameters on cellular physiology:
- $\beta$ = dilution due to cell growth; can vary ~10x
- $\alpha_0$ > 2-fold change thru cell cycle (gene dosage, Rb conc, etc)
  also strongly dependent on growth rate

➔ complex circuits usually cannot tolerate wildly floating operation points

➔ expect $[R]^*/K_R$ to be insensitive to parameters (i.e., homeostatic control) for large $n$ (cooperative repression)

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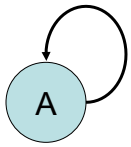
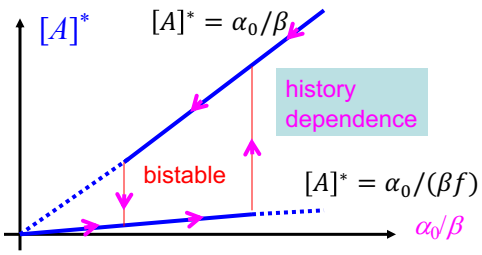
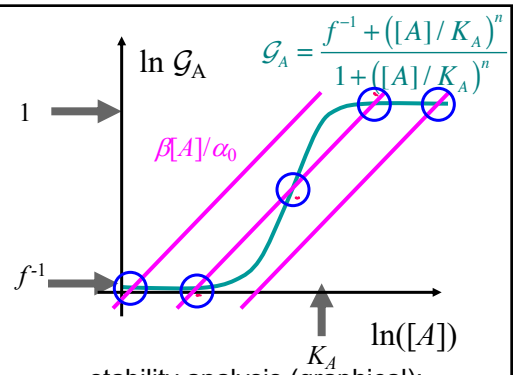
## 3. Positive autoregulation



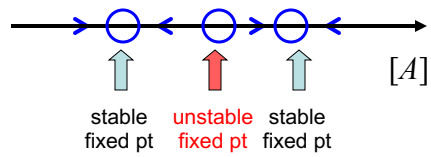

$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$

- large $\beta/\alpha_0$, $[A]^*$ ≈ basal level
- small $\beta/\alpha_0$, $[A]^*$ ≈ saturated level
- intermediate $\beta/\alpha_0$, $[A]^*$ has 3 soln




stability analysis (graphical):




➔ regime of bistability from the existence of unstable fixed point

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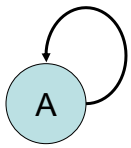
## 3. Positive autoregulation




$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$

stability analysis (analytic):

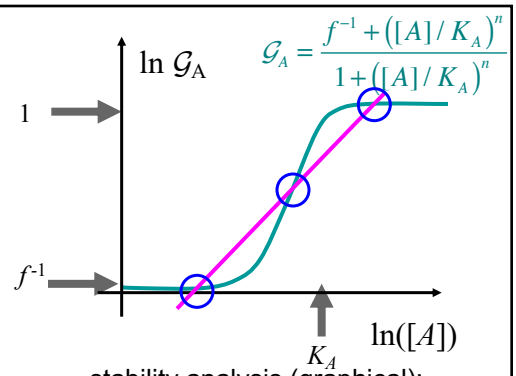
small perturbation: $\delta A \equiv [A] - [A]^*$

$$\frac{d}{dt}\delta A = \alpha_0 \left.\frac{d\mathcal{G}_A}{d[A]}\right|_{[A]^*} \cdot \delta A - \beta \cdot \delta A$$

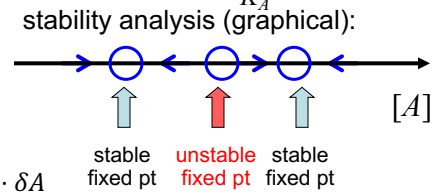
$$= \left(\alpha_0 \frac{\mathcal{G}_A}{[A]^*} s([A]^*) - \beta\right) \cdot \delta A = (s^* - 1)\beta \cdot \delta A$$

where $s^* \equiv \left.\frac{d \ln \mathcal{G}_A}{d \ln[A]}\right|_{[A]^*}$ is the "sensitivity"

➔ $s^* > 1$ for bistability



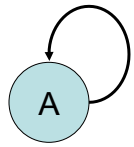

stability analysis (graphical):



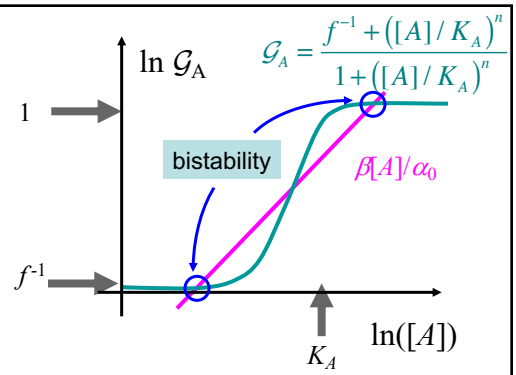

➔ regime of bistability from the existence of unstable fixed point

13

## 3. Positive autoregulation




$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$




➔ solve for regime of bistability (depends on $\sigma, f, n$ )

steady state: $\frac{p}{\sigma} = \frac{f^{-1} + p^n}{1 + p^n}$ where $p \equiv [A]/K_A; \sigma \equiv \alpha_0/(\beta K_A)$

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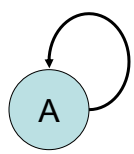
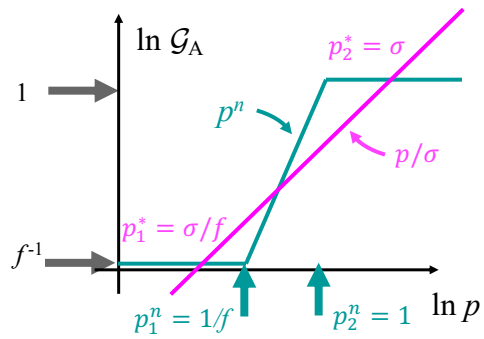
## 3. Positive autoregulation




$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$




➔ solve for regime of bistability (depends on $\sigma, f, n$ )

steady state: $\frac{p}{\sigma} = \frac{f^{-1} + p^n}{1 + p^n}$ where $p \equiv [A]/K_A; \sigma \equiv \alpha_0/(\beta K_A)$

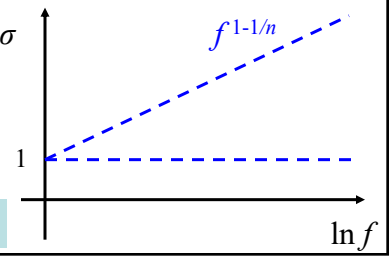
use approximate form of $\mathcal{G}_A$

bistability requires $p_1^* < p_1$ and $p_2^* > p_2$

$p_1^* = \sigma/f < p_1$ ➔ $\sigma < f^{1-1/n}$

$p_2^* = \sigma > p_2$ ➔ $\sigma > 1$

➔ bistability favored for $n \gg 1$ and $f \gg 1$

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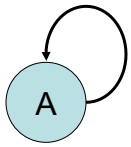
## 3. Positive autoregulation




$$\frac{d}{dt}[A] = \alpha \mathcal{G}_A([A]/K_A) - \beta[A]$$

typical parameters: $n = 2, f = 20$

➔ $1 \le \sigma \le f^{1-1/n} \approx 2$

(actual regime even narrower)

➔ need to fine tune parameters

➔ not robust to stochasticity, changes in growth conditions, or even cell cycle

➔ solve for regime of bistability (depends on $\sigma, f, n$ )

steady state: $\frac{p}{\sigma} = \frac{f^{-1} + p^n}{1 + p^n}$ where $p \equiv [A]/K_A; \sigma \equiv \alpha_0/(\beta K_A)$

use approximate form of $\mathcal{G}_A$

bistability requires $p_1^* < p_1$ and $p_2^* > p_2$

$p_1^* = \sigma/f < p_1$ ➔ $\sigma < f^{1-1/n}$

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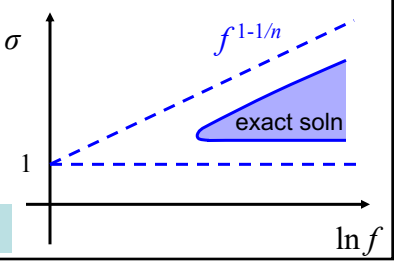
➔ bistability favored for $n \gg 1$ and $f \gg 1$

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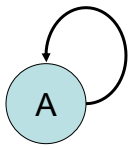
## 3. Positive autoregulation




$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$

typical parameters: $n = 2, f = 20$

➔ $1 \le \sigma \le f^{1-1/n} \approx 2$

(actual regime even narrower)

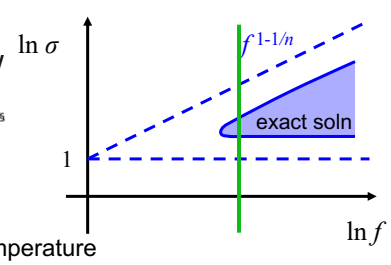
➔ need to fine tune parameters

➔ not robust to stochasticity, changes in growth conditions, or even cell cycle

**Prediction and measurement of an autoregulatory genetic module**

Farren J. Isaacs*†, Jeff Hasty†‡, Charles R. Cantor*, and J. J. Collins*§

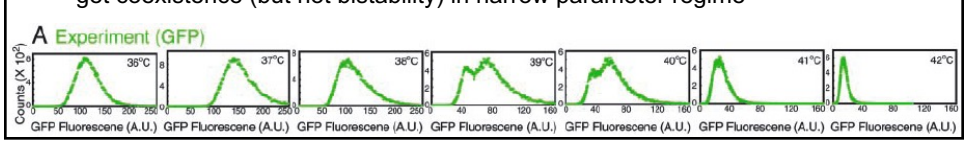
7714–7719 | PNAS | June 24, 2003 | vol. 100 | no. 13




-- activator = temperature sensitive mutant of CI

-- change $\beta$ (hence $\sigma = \alpha/(\beta K_A)$) by changing temperature

-- get coexistence (but not bistability) in narrow parameter regime

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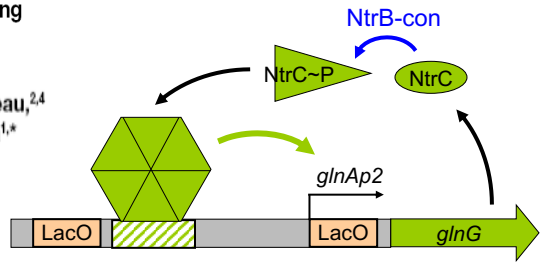
**Development of Genetic Circuitry Exhibiting Toggle Switch or Oscillatory Behavior in *Escherichia coli***

Mariette R. Atkinson,[1] Michael A. Savageau,[2,4] Jesse T. Myers,[2,3] and Alexander J. Ninfa[1,*]

Cell, Vol. 113, 597–607, May 30, 2003,

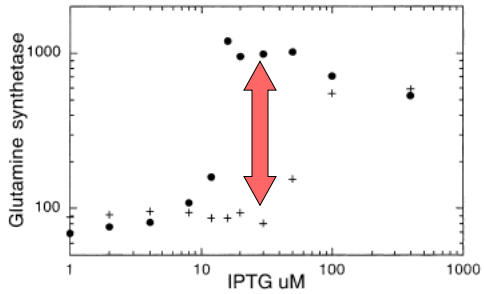
$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - \beta[A]$$




- use the highly cooperative Ntr-regulon of E. coli
- use mutant controller to insulate from cellular control
- add LacI control for tuning $\alpha_0$

➔ bistability (history-dependence) at intermediate IPTG levels

➔ makes use of special protein:
-- difficult to characterize
-- affects physiology

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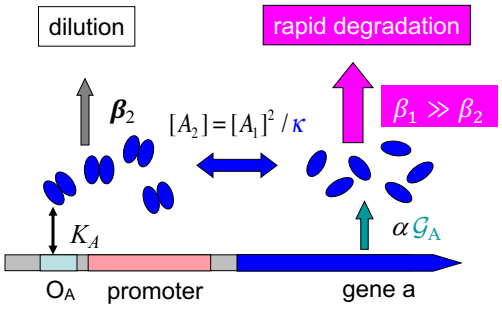
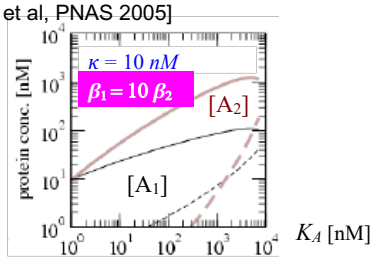
## Auto-activation by coop stability

[Buchler et al, PNAS 2005]




➔ high state dominated by dimers (instead of useless monomers)

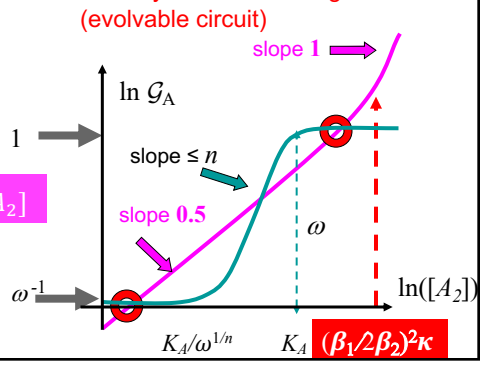
➔ bistability for a broad range of $K_A$ (evolvable circuit)

$$\frac{d}{dt}[A] = \alpha_0 \mathcal{G}_A([A]/K_A) - (\beta_1[A_1] + 2\beta_2[A_2])$$

steady state:

$$\alpha_0 \mathcal{G}_A([A]/K_A) = \beta_1\sqrt{\kappa[A_2]} + 2\beta_2[A_2]$$

➔ **bistability** may be achieved even with $n = 1$ and for small $\kappa$

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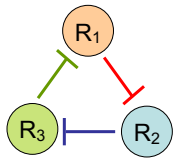
## 4. Oscillators

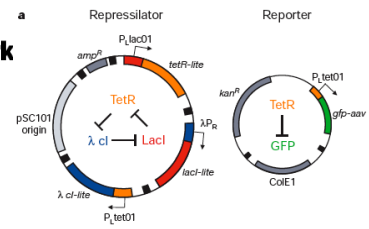



"Repressilator"

**A synthetic oscillatory network of transcriptional regulators**

Michael B. Elowitz & Stanislas Leibler

NATURE | VOL 403 | 20 JANUARY 2000 |



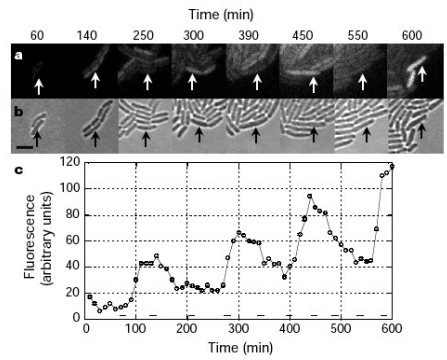

- a.k.a. ring-oscillator
- uses only transcriptional repressors (with protein degradation tags)
- modeling gives oscillation for sufficiently cooperative repression

$$\frac{d[R_1]}{dt} = \alpha_1 \cdot \mathcal{G}_{R1}([R_3]) - \beta_1 \cdot [R_1]$$

$$\frac{d[R_2]}{dt} = \alpha_2 \cdot \mathcal{G}_{R2}([R_1]) - \beta_2 \cdot [R_2]$$

$$\frac{d[R_3]}{dt} = \alpha_3 \cdot \mathcal{G}_{R3}([R_2]) - \beta_3 \cdot [R_3]$$

- oscillation observed; but noise abound
- not typically seen in bacteria or euk

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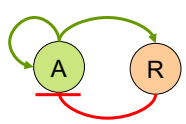
Predator-prey oscillators




$$\begin{cases} \dfrac{d[A]}{dt} = \alpha \cdot \mathcal{G}_A([A]) \cdot \mathcal{G}_R([R]) - \beta \cdot [A] \\ \dfrac{d[R]}{dt} = \alpha \cdot \mathcal{G}_A([A]) - \beta \cdot [R] \end{cases}$$

linear stability analysis around $[A]^*, [R]^*$, such that $\begin{cases} \alpha \cdot \mathcal{G}_A([A]^*) \cdot \mathcal{G}_R([R]^*) = \beta \cdot [A]^* \\ \alpha \cdot \mathcal{G}_A([A]^*) = \beta \cdot [R]^* \end{cases}$

let $\delta A \equiv [A] - [A]^*, \delta R \equiv [R] - [R]^*$

then $\dfrac{d}{dt}\begin{bmatrix}\delta A \\ \delta R\end{bmatrix} = \beta \begin{bmatrix} s_A^* - 1 & -s_R^*[A]^*/[R]^* \\ s_A^*[R]^*/[A]^* & -1 \end{bmatrix} \cdot \begin{bmatrix}\delta A \\ \delta R\end{bmatrix}$

where $s_A^* = \left.\dfrac{d \ln \mathcal{G}_A}{d \ln[A]}\right|_{[A]^*}$, $\quad s_R^* = -\left.\dfrac{d \ln \mathcal{G}_R}{d \ln[R]}\right|_{[R]^*}$

try $\delta A \sim e^{\lambda t}, \delta R \sim e^{\lambda t}$, $\quad$ get $\lambda = \dfrac{\beta}{2}\left[s_A^* - 2 \pm \sqrt{(s_A^* - 2)^2 - 4 s_A^* s_R^*}\right]$

$$\begin{cases} \mathrm{Im}\{\lambda\} = 0 & \text{no oscillation} \\ \mathrm{Im}\{\lambda\} \neq 0,\ \mathrm{Re}\{\lambda\} < 0 & \text{damped oscillation} \\ \mathrm{Im}\{\lambda\} \neq 0,\ \mathrm{Re}\{\lambda\} > 0 & \text{amplifying oscillation} \end{cases}$$

$\Leftarrow \quad s_A^* > 2,\ s_R^* > (s_A^* - 2)^2/(4 s_A^*)$

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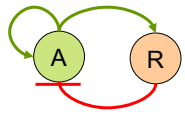
Predator-prey oscillators

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solve for regime of oscillation:

assuming that instability occurs for $s_A^* > 2$ and $s_R^* > (s_A^* - 2)^2/(4s_A)$

then $\mathcal{G}_A \approx ([A]/K_A)^{n_A}$ for $f_A^{-1/n_A} < [A]/K_A < 1$,

$\mathcal{G}_R \approx ([R]/K_R)^{-n_R}$ for $f_R^{1/n_R} > [R]/K_R > 1$

steady-state (with $\sigma = \alpha/\beta K$, taking $K_A = K_R$ for simplicity):

$$\begin{cases} \sigma \cdot ([A]^*/K)^{n_A} = [R]^*/K \\ \sigma \cdot ([A]^*/K)^{n_A} \cdot ([R]^*/K)^{-n_R} = [A]^*/K \end{cases}$$

soln exist if $n_R > 1$, and $1 < \sigma < \min\left\{ f_A^{1+1/[n_A \cdot (n_R - 1)]}, f_R^{[1+n_A \cdot (n_R - 1)]/n_R} \right\}$

for large $n_A$ and $n_R$, unstable (osc) regime is $1 < \sigma < \min\left\{ f_A, f_R^{n_A} \right\}$

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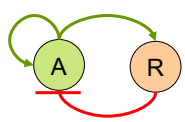
Predator-prey oscillators

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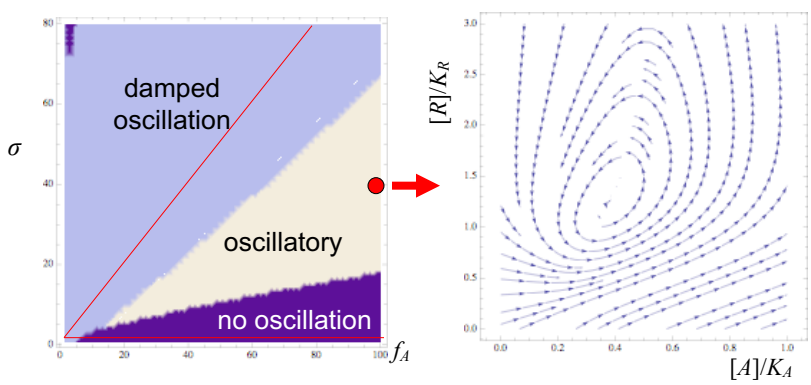
phase diagram ($f_A = f_R = 100$, $n_A = n_R = 4$)

stream plot

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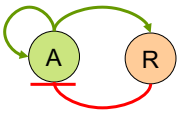
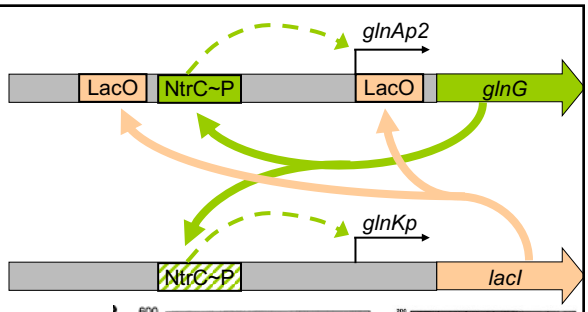
25

Predator-prey oscillators

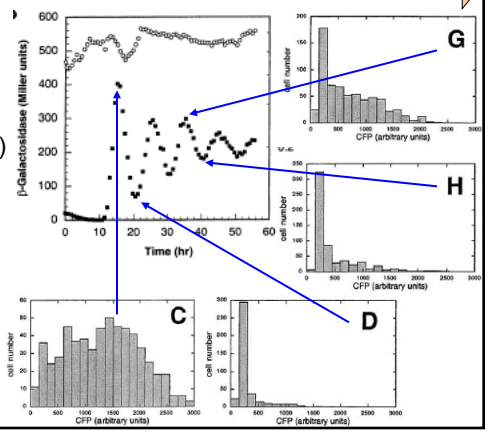



Development of Genetic Circuitry Exhibiting Toggle Switch or Oscillatory Behavior in *Escherichia coli*

Mariette R. Atkinson,[1] Michael A. Savageau,[2,4] Jesse T. Myers,[2,3] and Alexander J. Ninfa[1,*]

Cell, Vol. 113, 597–607, May 30, 2003,




- uses transcriptional activator (NtrC on $\sigma^{54}$) and repressors (LacI)
- population shows up to 4 cycles
- damped oscillation (LacI-CFP fusion)

$$\frac{d[R]}{dt} = \alpha_R \cdot \mathcal{G}_{A1}\left(\frac{[A]}{K_A}\right) - \beta_R \cdot [R]$$

$$\frac{d[A]}{dt} = \alpha_A \cdot \mathcal{G}_{A2}\left(\frac{[A]}{K_A}\right) \cdot \mathcal{G}_R\left(\frac{[A]}{K_R}\right) - \beta_A \cdot [A]$$

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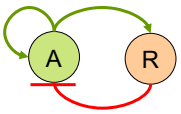
Predator-prey oscillators




- amplitude & period of oscillation not determined by stability analysis
- typically period controlled by a slow step = relaxational oscillator
- amplitudes by binding affinities

**Circadian clocks limited by noise**

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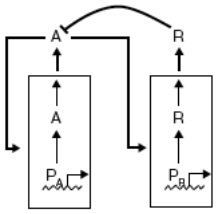
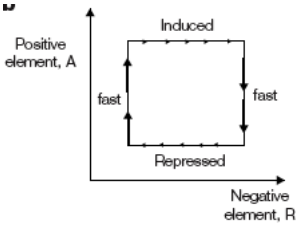
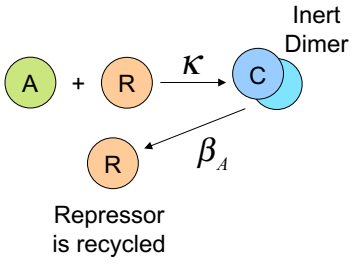
Naama Barkai, Stanislas Leibler

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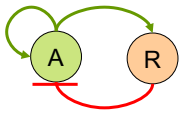
Predator-prey oscillators

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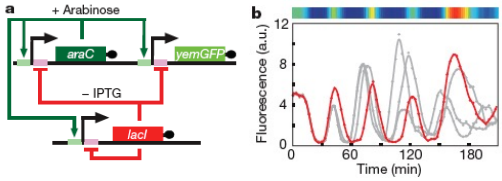
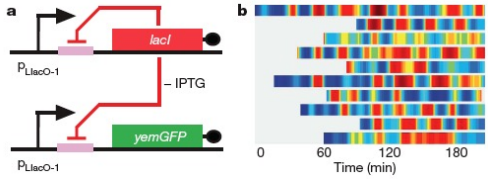
Vol 456|27 November 2008|doi:10.1038/nature07389

**A fast, robust and tunable synthetic gene oscillator**

Jesse Stricker[1]*, Scott Cookson[1]*, Matthew R. Bennett[1,2]*, William H. Mather[1], Lev S. Tsimring[2] & Jeff Hasty[1,2]




activation not even necessary!

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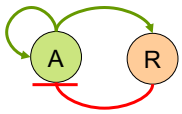
Predator-prey oscillators

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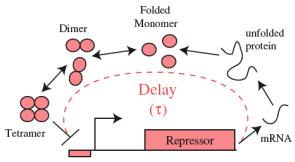
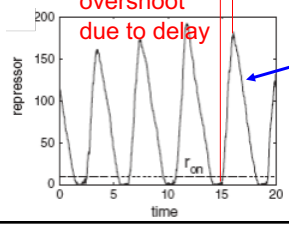



delay in repression crucial

$$\frac{d[R]}{dt} = \alpha \cdot \mathcal{G}_R\left(\frac{[R]_\tau}{K}\right) - \underbrace{\gamma \cdot \frac{\cancel{[R]}}{[R]+R_0}}_{\text{saturated proteolysis}}$$

$\tau$

overshoot due to delay




$$\frac{d[R]}{dt} = -\gamma$$

linear discharge (slow)

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