**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
A. Models and behaviors of simple genetic circuits

1. Model of gene expression:

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

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

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

mean-field description (rate eqn) for gene $i$

$$\frac{dg_i}{dt} = \alpha_{m,i}[g_i] - \beta_{m,i}[m_i]$$

$$\frac{dp_i}{dt} = \alpha_{p,i}[m_i] - (\beta_p + \lambda)[p_i]$$

- $\lambda$: growth rate

- All parameters growth-rate dependent (next major topic); fixed for now.
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 \([m_i^-] = \alpha_{m,0} \mathcal{P}_i / \beta_{m,i} = m_{0,i} \cdot \mathcal{R}\)

\[ m_{0,i} = \frac{\alpha_{m,0} [\text{RNAP}]_{\text{av}}}{\beta_{m,i} K_{P,i}} \]

\( \mathcal{R} \) 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 \([m_i^-] = \alpha_{m,0} \mathcal{P}_i / \beta_{m,i} = m_{0,i} \cdot \mathcal{R}\)

\[ m_{0,i} = \frac{\alpha_{m,0} [\text{RNAP}]_{\text{av}}}{\beta_{m,i} K_{P,i}} \]
in terms of protein dynamics \( \frac{d}{dt} [p_i] = \alpha_i - \beta_i \cdot [p_i] \)
\[
\alpha_i = \alpha_{p,i} \cdot [m_i] = \alpha_{p,i} \cdot m_{0,i} \cdot R_i = \alpha_{0,i} \cdot G_i \\
\beta_i = \beta_{p,i} + \lambda \\
\text{max rate: } \dot{G}_i = \frac{R_i}{\max(R_i)}
\]

consider tsx init control only (for simplicity), with \([m_i]^* = \alpha_{m,0} P_i / \beta_{m,i} = m_{0,i} \cdot R\)
\[
m_{0,i} = \frac{\alpha_{m,0} [RNA_P]_{av}}{\beta_{m,i}} \quad \ell
\]

\( \ln G_A = \frac{f_A^{-1} + \left(\frac{[A]}{K_A}\right)^{n_A}}{1 + \left(\frac{[A]}{K_A}\right)^{n_A}} \quad \text{capacity: } f_A \)

\( \ln G_R = \frac{1 + f_R^{-1} \left(\frac{[R]}{K_R}\right)^{n_R}}{1 + \left(\frac{[R]}{K_R}\right)^{n_R}} \quad \text{capacity: } f_R \)

2. Negative autoregulation (a very common network motif)

\[
\frac{d}{dt} [R] = \alpha_0 G_R ([R] / K_R) - \beta [R] \\
\]

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 \( \sim 10^x \)
  - \( \alpha_0 = 2\)-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)
3. Positive autoregulation

\( \frac{d}{dt} [A] = \alpha_0 G_A([A]/K_A) - \beta [A] \)

- large \( \beta \alpha_0 \), \([A]^*\) = basal level
- small \( \beta \alpha_0 \), \([A]^*\) = saturated level
- intermediate \( \beta \alpha_0 \), \([A]^*\) has 3 soln

\([A]^* = \frac{\alpha_0}{\beta} \)

Regime of bistability from the existence of unstable fixed point

Stability analysis (analytic):

\[ s^* \equiv \frac{d \ln G_A}{d \ln [A]} \]

is the "sensitivity"

\( s^* > 1 \) for bistability
3. Positive autoregulation

\[ \frac{d}{dt} [A] = \alpha_0 G_A([A]/K_A) - \beta [A] \]

\[ \ln G_A = \frac{f^{-1} + ([A]/K_A)^n}{1 + ([A]/K_A)^n} \]

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

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

\[ \ln \sigma \]

\[ f^{-1/n} \]

\[ \ln f \]

bistability favored for \( n \gg 1 \) and \( f \gg 1 \)
3. Positive autoregulation

\[ \frac{d}{dt}[A] = a_G A[A] / K_A - \beta[A] \]

- typical parameters: \( n = 2, f = 20 \)
  - \( 1 \leq \sigma \leq f^{1-1/n} \approx 2 \) (actual regime even narrower)
  - need to fine tune parameters
  - not robust to stochasticity, changes in growth conditions, or even cell cycle

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

steady state: \( p = f^{-1} + p^n \) where \( p \equiv [A]/K_A \); \( \sigma \equiv a_0/(\beta K_A) \)

use approximate form of \( G_A \)

bistability requires \( p_1^* < p_1 \) and \( p_2^* > p_2 \)

- \( p_1^* = \sigma / f < p_1 \rightarrow \sigma < f^{1-1/n} \)
- \( p_2^* = \sigma > p_2 \rightarrow \sigma > 1 \)

\( \rightarrow \) bistability favored for \( n \gg 1 \) and \( f > 1 \)

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Prediction and measurement of an autoregulatory genetic module

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

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

Mariette R. Atkinson, Michael A. Savageau, Jesse T. Myers, and Alexander J. Ninfa


\[ \frac{d}{dt}[A] = \alpha_0 S_A ([A]/K_A) - \beta[A] \]

- 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

### Auto-activation by coop stability

\[ \frac{d}{dt}[A] = \alpha_0 S_A ([A]/K_A) - \beta_1[A] \]

steady state:
\[ \alpha_0 S_A ([A]/K_A) = \beta_1 \kappa [A_2] + 2 \beta_2 [A_2] \]

**bistability** may be achieved even with \( n = 1 \) and for small \( \kappa \)**

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\[ \frac{d}{dt}[A] = \alpha_1 [A]/K_A - \beta_1 [A] \]

steady state:
\[ \alpha_1 [A]/K_A = \beta_1 \kappa [A_2] + 2 \beta_2 [A_2] \]

**bistability** may be achieved even with \( n = 1 \) and for small \( \kappa \)**
4. Oscillators

"Repressilator"

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

\[
\begin{align*}
\frac{d[R_1]}{dt} &= \alpha_1 \cdot G_{R_1}(\{R_1\}) - \beta_1 \cdot [R_1] \\
\frac{d[R_2]}{dt} &= \alpha_2 \cdot G_{R_2}(\{R_2\}) - \beta_2 \cdot [R_2] \\
\frac{d[R_3]}{dt} &= \alpha_3 \cdot G_{R_3}(\{R_3\}) - \beta_3 \cdot [R_3]
\end{align*}
\]

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

Predator-prey oscillators

\[
\begin{align*}
\frac{d[A]}{dt} &= \alpha \cdot G_A(\{A\}) \cdot G_R(\{R\}) - \beta \cdot [A] \\
\frac{d[R]}{dt} &= \alpha \cdot G_R(\{A\}) - \beta \cdot [R]
\end{align*}
\]

linear stability analysis around \([A]^*, [R]^*\), such that

\[
\begin{align*}
\alpha \cdot G_A(\{A\}) \cdot G_R(\{R\}) &= \beta \cdot [A]^* \\
\alpha \cdot G_R(\{A\}) &= \beta \cdot [R]^*
\end{align*}
\]

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

then

\[
\begin{align*}
\frac{d}{dt} \frac{\delta A}{\delta R} &= \beta \begin{bmatrix}
-1 & s_R^* \frac{[A]^*}{[R]^*} \\
\frac{d \ln G_A}{d \ln [A]}(\{A\}) & -s_A^* \frac{[R]^*}{[A]^*}
\end{bmatrix}
\end{align*}
\]

where

\[
\begin{align*}
\delta A &= \frac{d \ln G_A}{d \ln [A]}(\{A\}) \\
\delta R &= \frac{d \ln G_R}{d \ln [R]}(\{R\})
\end{align*}
\]

try \(\delta A \sim e^{\lambda t}, \delta R \sim e^{\lambda t}\)

get \(\lambda = \frac{\beta}{2} \left[ s_A^* - 2 \pm \sqrt{(s_A^* - 2)^2 - 4s_A^*s_R^*} \right]\)

\[
\begin{align*}
\text{Im} \{\lambda\} &= 0 & \text{no oscillation} \\
\text{Im} \{\lambda\} \neq 0, \text{Re} \{\lambda\} < 0 & \text{damped oscillation} \\
\text{Im} \{\lambda\} \neq 0, \text{Re} \{\lambda\} > 0 & \text{amplifying oscillation}
\end{align*}
\]

\(s_A^* > 2, s_R^* > (s_A^* - 2)^2 / (4s_A^*)\)
Predator-prey oscillators

\[
\begin{align*}
\frac{d[A]}{dt} &= \alpha \cdot G_A([A]) \cdot G_R([R]) - \beta \cdot [A] \\
\frac{d[R]}{dt} &= \alpha \cdot G_A([A]) - \beta \cdot [R]
\end{align*}
\]

solve for regime of oscillation:

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

then \( G_A = ([A] / K_A)^{s_A^*} \) for \( f_A^{1/s_A} < [A] / K_A < 1 \),

\( G_R = ([R] / K_R)^{s_R^*} \) for \( f_R^{1/s_R} > [R] / K_R > 1 \)

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

\[
\begin{align*}
\sigma \cdot ([A]/K)^{s_A} &= [R]/K \\
\sigma \cdot ([A]/K)^{s_A} \cdot ([R]/K)^{-s_R} &= [A]/K
\end{align*}
\]

so \( n_A > 1, \) and \( 1 < \sigma < \min \left\{ \frac{1}{f_A^{1/[s_A]}}, \frac{1}{f_R^{1/[s_R]}}, \frac{1}{f_A^{1/[s_A]}/[R]} \right\} \)

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

Predator-prey oscillators

\[
\begin{align*}
\frac{d[A]}{dt} &= \alpha \cdot G_A([A]) \cdot G_R([R]) - \beta \cdot [A] \\
\frac{d[R]}{dt} &= \alpha \cdot G_A([A]) - \beta \cdot [R]
\end{align*}
\]

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

stream plot

for large \( n_A \) and \( n_R \), unstable (osc) regime is \( 1 < \sigma < \min \left\{ f_A^{1/[s_A]}, f_R^{1/[s_R]} \right\} \)
 Predator-prey oscillators

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

\[
\frac{d[R]}{dt} = \alpha_R \cdot \frac{[A]}{K_A} - \beta_R \cdot [R]
\]

\[
\frac{d[A]}{dt} = \alpha_A \cdot \frac{[A]}{K_A} \cdot \frac{[A]}{K_A} - \beta_A \cdot [A]
\]

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

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Circadian clocks limited by noise

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

A *fast, robust and tunable synthetic gene oscillator*

Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty

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