**Topic 4: Genetic Circuits**

A. **Integrated model of gene expression**
   1. constitutive gene expression
   2. transcription control
   3. translation control and mRNA stability
   4. control of protein degradation

B. **Simple circuits using only transcriptional control**
   1. negative autoregulation
   2. positive autoregulation
   3. toggle switch
   4. oscillators

C. **Noise in gene expression**

D. **Metabolic control**
   1. gene regulation
   2. effect of inducer
   3. metabolic feedback
A. Integrated model of gene expression

1. Constitutive gene expression:

- **steady-state mRNA level:**
  \[ [m_i] = [g_i] \cdot \frac{\alpha_m}{\beta_m} \sim \text{few/cell} \]

- **steady-state protein level:**
  \[ [p_i] = [p_i] \cdot \frac{\alpha_p}{\beta_p} \sim 1000/cell \]

\[ g = \# \text{ promoters per cell; 1-8/cell for chromosomal promoters; can be >100 on plasmids} \]
\[ \alpha_m = \text{mRNA synthesis rate per promoter; max \sim 1/sec; typical \sim 1/min} \]
\[ \beta_m^{-1} = \text{mRNA life time; typical 1~2 min; max \sim doubling time} \]
\[ \alpha_p = \text{protein synthesis rate per mRNA; typical high \sim 10/min} \]
\[ \beta_p^{-1} = \text{protein life time; max \sim doubling time; short \sim min} \]

- **mean-field description (rate eqn) for gene** \( i \)
  \[
  \frac{d}{dt} [m_i] = \alpha_m [g_i] - \beta_m [m_i] \\
  \frac{d}{dt} [p_i] = \alpha_p [m_i] - \beta_p [p_i]
  \]

  \[ \text{"translational efficiency"} \]

- **not contained in the simple model:**
  - delay from txs init to completion of mRNA synthesis
    [typically \sim 0.5 min, could be longer with roadblocks, txs 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 \( V_{\text{cell}}(t) \)
    - gene dose change (differential increase of \( g(t) \) and \( V_{\text{cell}}(t) \) 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 \( \leq \) cell doubling time
    [very short-time dynamics okay; e.g., allosteric control, protein modifications]
  - for time scales \( \gg \) cell doubling time, parameters are well-defined in **exponential growth phase**
    can set \( \frac{d}{dt} [p_i] = \alpha_i [g_i] - \beta_i [p_i] \) where \( \alpha_i = \alpha_m \cdot \alpha_p / \beta_m \)
    [note: \( \alpha_p / \beta_m \sim \# \text{ transcripts per mRNA life time = "burstiness" of mRNA } i \)]
  - all parameters **growth-rate dependent** (next major topic); fixed for now.
2. transcriptional control

- transcription initiation control \( \alpha_m = \alpha_{m,0}P_{\text{promoter}}(\text{TF}_1, \text{TF}_2, \ldots) \)
- transcription termination control (intrinsic terminators)

\[
\alpha_m = \alpha_{m,0}P_{\text{promoter}} (1 - P_T) = \alpha_{m,0}P_{\text{promoter}} (1 - P_{T1}) (1 - P_{T2}) \cdots
\]

- tsx termination: stabilized by protein, sRNA or stalled ribosome
- anti-termination: default stably by protein or sRNA

- quantified by \( P_T \): “probability” for terminator formation [need to form within a certain time window]
- multiple terminators: \( P_{T1}, P_{T2}, \ldots \)
  \[
P_T = P_{T1} + (1 - P_{T1}) P_{T2} + \ldots
\]
- overall mRNA synthesis rate multiplicative:

\[
\alpha_m = \alpha_{m,0}P_{\text{promoter}} (1 - P_T) = \alpha_{m,0}P_{\text{promoter}} (1 - P_{T1}) (1 - P_{T2}) \cdots
\]

3. translational control and mRNA stability

- Shine-Dalgarno (SD) seq: \texttt{AGGAGGNNNNNNAUG} [strong translation from 4 consecutive matches + appropriate spacing to AUG]
- key control: access of ribosome to ribosomal binding site (RBS)

\[
\alpha_p = \alpha_{p,0}P_{\text{RBS}}(\text{regulator})
\]

- analogous to tsx initiation control
- tunable over 1000-fold (but not heavily used by endogenous genes)
- untranslated mRNA degraded rapidly (use it or lose it!)

mRNA stability (\( \beta_m \)): see Topics 3 for small RNA mediated gene silencing
4. Control of protein degradation

- Protein turnover can depend on the state of protein (e.g., protein usage or formation of protein complex).

degradation rate of free protein \( p_f \): \( \beta p_f \)
degradation rate of protein in complex \( p_c \): \( \beta p_c \)

- Stabilizing complex (e.g., r-protein in ribosome): \( \beta p_c \ll \beta p_f \)
- Opposite case for complex with adaptor molecules of protease.

Model: let conc of the free form of partner protein be \( P' \)

\[
\frac{d}{dt} P + \frac{k_p}{k_d} PP' = \begin{cases} 
\frac{d}{dt} p_f & = \alpha - \beta p_f - k_1 p_f + k_2 p_c \\
\frac{d}{dt} p_c & = -\beta p_c + k_1 p_f - k_2 p_c 
\end{cases}
\]

Steady-state: \( \frac{p_f}{p_c} = \frac{k_2 + \beta p_c}{\beta p_f} \) \( \iff \) effective dissociation constant \( k = \frac{k_2}{k_1} \)

- Total conc of \( P \) (\( p = p_f + p_c \)):

\[
\frac{d}{dt} p = \alpha - \left( \frac{\beta p_f + \beta p_c p'}{1 + p'} \right) \cdot p
\]

Altogether,

\[
\frac{d}{dt} p = \alpha (A, B, C, ...) - \beta (A', B', C', ...) \cdot p
\]

B. Simple circuit using transcriptional control

Consider tsx init control only (for simplicity), with \( \alpha = \alpha_0 G \)

\[
G_A = f_A^{-1} + \left( \frac{[A]}{K_A} \right)^n_A \\
G_R = 1 + \frac{\left( [R]/K_R \right)^n_R}{1 + \left( [R]/K_R \right)^n_R}
\]

\[
K_A = \ln([A]) \\
K_R = \ln([R])
\]

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1. Negative autoregulation
(a very common network motif)

\[ \frac{d[R]}{dt} = \alpha_R \frac{G_R}{K_R} - \beta_0[R] \]

assume circuit ‘properly’ biased: \( K_R > [R]^* > f_R^{1/n} K_R \) or \( K_R < \alpha_0/\beta_0 < K_R f_R^{1/n} \)

steady-state solution: \( \frac{[R]^*}{K_R} = \left( \frac{\alpha_R}{\beta_0 K_R} \right)^{1/(n+1)} \)

- general dependence of parameters on cellular physiology:
  - \( \beta_0 = \) 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 if \( n \) is large
  \( \approx \) homeostatic control

2. Positive autoregulation

\[ \frac{d[A]}{dt} = \alpha_A \frac{G_A}{K_A} - \beta_0[A] \]

- large \( \beta_0/\alpha_0 \), \( [A]^* \approx \) basal level
- small \( \beta_0/\alpha_0 \), \( [A]^* \approx \) saturated level
- intermediate \( \beta_0/\alpha_0 \), \( [A]^* \) has 3 solns

\[ [A]^* = \alpha_0/\beta_0 \]

regime of bistability from the existence of unstable fixed point
2. Positive autoregulation

\[ \frac{d[A]}{dt} = \alpha_o G_A \left( \frac{[A]}{K_A} \right) - \beta_o [A] \]

stability analysis (analytic):

\[ \frac{d}{dt} \delta A = \alpha_o \left. \frac{\partial G_A}{\partial [A]} \right|_{[A]} \cdot \delta A - \beta_o \cdot \delta A \]

where \( s^* = \left. \frac{\partial \ln G_A}{\partial \ln [A]} \right|_{[A]} \) is "sensitivity".

\[ s^* > 1 \] for bistability

\[ \text{solve for regime of bistability} \quad (\text{depends on } \sigma, f, n) \]

steady-state: \( p = \frac{f^{-1} + (p \sigma)^n}{1 + (p \sigma)^n} \) where \( p = [A] \beta_o / \alpha_o, \sigma = \alpha_o / (\beta_0 K_A) \)
2. Positive autoregulation

\[ \frac{d[A]}{dt} = \alpha_0 \frac{G_A [A]}{K_A} - \beta_0 [A] \]

\[ G_A = (\alpha p)^n \]

- solve for regime of bistability (depends on \( \sigma, f, n \))
- steady-state: \( p = \frac{f^{-1} + (p\sigma)^n}{1 + (p\sigma)^n} \)
- use approximate form of \( G_A \)
- at \( G_A(p_1) = 1/f \) or \( p_1 = 1/(\sigma f^{1/n}) \)
- bistability if \( p^* = 1/f < p_1 \Rightarrow \sigma < f^{-1/n} \)
- at \( G_A(p_2) = 1/\sigma \) or \( p_2 = 1/\sigma \)
- bistability if \( p^* = 1 > p_2 \Rightarrow \sigma > 1 \)

\[ p^* \equiv \frac{\beta_0}{\alpha_0}, \sigma \equiv \frac{\alpha_0}{\beta_0 K_A} \]

\[ \text{bistability requires } n > 1 \text{ and } f \gg 1 \]

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

\[
\frac{d[A]}{dt} = \alpha_0 G_A \left( \frac{[A]}{K_A} \right) - \beta_0 [A]
\]

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

---

\( \sigma = \alpha_0 \left/ \left( \beta_0 K_A \right) \right. \)

---

\( \ln \sigma = \ln f - \frac{1-1/n}{n} \)

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

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

\[
\frac{d[A]}{dt} = \alpha_0 G_A \left( \frac{[A]}{K_A} \right) - \beta_0 [A]
\]

- 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

\[ \frac{d[A]}{dt} = \alpha_0 \cdot G_A \left( \frac{[A_2]}{K_A} \right) - \left( \beta_1 \cdot [A_1] + 2\beta_2 \cdot [A_2] \right) \]

steady-state soln:
\[ \alpha_0 \cdot G_A \left( \frac{[A_2]}{K_A} \right) = \beta_1 \left( K_A \right) + 2\beta_2 \cdot [A_2] \]

\[ \rightarrow \text{bistability may be achieved even with } n = 1 \text{ and for small } \kappa \]

\[ k = 10 \text{nM} \]
\[ \beta_1 = 10 \beta_2 \]

\[ A_1 \]
\[ A_2 \]

\[ K_A \]

\[ \kappa \]

\[ \text{high state dominated by dimers (instead of useless monomers)} \]
\[ \rightarrow \text{bistability for a broad range of } K_A \text{ (evolvable circuit)} \]

\[ \ln G_A \]
\[ \ln([A_2]) \]

\[ \text{slope } 1 \]
\[ \text{slope } \leq n \]
\[ \text{slope 0.5} \]
\[ \omega \]
\[ \omega^{-1} \]

\[ K_A \cdot \omega^{1/n} \]

\[ (\beta_1/2\beta_2)^2 \kappa \]

4. Oscillators

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler
NATURE | VOL 405 | 20 JANUARY 2000

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

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

\[ \text{oscillation observed; but noise abound} \]
\[ \text{not typically seen in bacteria or euk} \]
Predator-prey oscillators

\[
\frac{d[A]}{dt} = \alpha \cdot G_A([A]) \cdot G_A([R]) - \beta \cdot [A] \\
\frac{d[R]}{dt} = \alpha \cdot G_A([A]) - \beta \cdot [R]
\]

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

\[
\alpha \cdot G_A([A]^*) \cdot G_A([R]^*) = \beta \cdot [A]^* \\
\alpha \cdot G_A([A]^*) = \beta \cdot [R]^*
\]

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

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

where \(s_A^* = \frac{\partial \ln G_A}{\partial \ln [A]}_{[A]^*}, \quad s_R^* = \frac{\partial \ln G_R}{\partial \ln [R]}_{[R]^*} \)

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

- \(\text{Im} \{\lambda\} = 0 \quad \text{no oscillation} \)
- \(\text{Im} \{\lambda\} \neq 0, \text{Re} \{\lambda\} < 0 \quad \text{damped oscillation} \)
- \(\text{Im} \{\lambda\} \neq 0, \text{Re} \{\lambda\} > 0 \quad \text{amplifying oscillation} \)

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

Predator-prey oscillators

\[
\frac{d[A]}{dt} = \alpha \cdot G_A([A]) \cdot G_A([R]) - \beta \cdot [A] \\
\frac{d[R]}{dt} = \alpha \cdot G_A([A]) - \beta \cdot [R]
\]

solve for regime of oscillation:

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

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

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

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

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

soln exist if \(n_A > 1, \quad 1 < \sigma < \min \left\{ f_A^{1/n_A (s_A^* - 1)}, f_R^{1/n_A (s_R^* - 1)/s_A^*} \right\} \)

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

\[
\frac{d[A]}{dt} = \alpha \cdot G_A([A]) \cdot G_R([R]) - \beta \cdot [A]
\]

\[
\frac{d[R]}{dt} = \alpha \cdot G_A([A]) - \beta \cdot [R]
\]

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 \(1 < \sigma < \min\left\{f_A, f_R\right\}\)

Predator-prey oscillators

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

Mariette R. Atkinson,¹ Michael A. Savageau,²¼ Jesse T. Myers,²⁴ and Alexander J. Ninfa³


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

\[
\frac{d[R]}{dt} = \alpha_R \cdot G_R \left( \frac{[A]}{K_R} \right) - \beta_R \cdot [R]
\]

\[
\frac{d[A]}{dt} = \alpha_A \cdot G_A \left( \frac{[A]}{K_A} \right) \cdot G_R \left( \frac{[A]}{K_R} \right) - \beta_A \cdot [A]
\]

lacI

\(glnAp2\)

\(glnAp2\)

\(glnG\)

\(glnKp\)
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

A fast, robust and tunable synthetic gene oscillator

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activation not even necessary!
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. Marler†, Lev S. Tsimring‡ & Jeff Hardy*†

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Delay in repression crucial

\[ \frac{d[R]}{dt} = \alpha \cdot G_R \left( \frac{[R]}{K} \right) - \gamma \cdot \frac{[R]}{[R] + R_0} \]

Overshoot due to delay

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

Saturated proteolysis

Linear discharge (slow)