Central Dogma + regulation

- tRNA
- rRNA
- sRNA
- mRNA
- transcription
- ribosomal proteins
- structural proteins
- transporters
- enzymes
- regulators

 regulatory proteins
replication
mRNA translation
- DNAp
- RNAp
- structural proteins
- transporters
- enzymes
- regulators

- amino acids, NTP, dNTP, lipids, ...
- sugar, NH3, O2
- coupled to environmental signals; coord growth program

tsx initiation control by transcription factors (TF)
tsl initiation control by sRNA and RNA-binding proteins
tsx termination control by sRNA and anti-terminators
control of mRNA and protein degradation
control of enzyme activity by metabolites

Integrated model of gene expression

1. Constitutive gene expression:

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

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

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

\[ \frac{d}{dt}[m_i] = \alpha_{m,i}[g_i] - \beta_{m,i}[m_i] \]
\[ \frac{d}{dt}[p_i] = \alpha_{p,i}[m_i] - \beta_{p,i}[p_i] \]

10-30 proteins/mRNA
("burstiness")

\[ [g_i]^* = [m_i]^* \cdot \frac{\alpha_p}{\beta_p} ~ 1000/\text{cell} \]
transcriptional initiation and termination in bacteria

tsx init control by activators, repressors

Basic Models of Transcriptional Control

1. tsx init by RNAP alone

\[
\begin{align*}
\text{RNAP + promoter} & \xrightarrow{k_p} \text{RNAP} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAP} + \text{promoter} + \text{mRNA} \\
[P] & \quad [g] & \quad [m]
\end{align*}
\]

• mRNA level:

\[
\frac{d}{dt} [m] = \alpha P [g] - \beta [m]
\]

mRNA degradation

probability of promoter occupation by RNAP

• steady-state mRNA level (measurable):

\[
[m]^* = \frac{\alpha P [g]}{\beta}
\]

• from protein-DNA interaction:

\[
P = \frac{[P]_{av}}{[P]_{av} + K_p}
\]

⇒ for RNAP by itself,

\[
P \approx \frac{[P]_{av}}{K_p} \ll 1
\]

⇒ Transcription factors (TF) can modulate \(P\) or \(\alpha\)
2. Activation by recruitment

How does gene expression depend on the level of TF, [A]?

Strategy: [Shea & Ackers, 1985]
-- assume $[m]^* = \alpha \mathcal{P}(A, [P]) [g]/\beta$
-- $\mathcal{P}$ computed according to thermodynamics (assumes thermal equilibrium)

For operator site alone: $P_A = \frac{[A]}{[A] + K_A}$

**Total** probability of RNAp binding to promoter in the presence of A:

$$
\mathcal{P}(A, [P]) = \frac{W(0,1) + W(1,1)}{W(0,0) + W(0,1) + W(1,0) + W(1,1)}
$$

where $W(\sigma_A, \sigma_P) = \text{weight of}
\begin{cases} 
\text{operator A is occupied (}\sigma_A = 1\text{)} \text{ or unoccupied (}\sigma_A = 0\text{)} \\
\text{promoter is occupied (}\sigma_P = 1\text{)} \text{ or unoccupied (}\sigma_P = 0\text{)}
\end{cases}$

## Dependence of the total probability of RNAp-promoter binding on A:

$$
\mathcal{P}(A, [P]) = \frac{W(0,1) + W(1,1)}{W(0,0) + W(0,1) + W(1,0) + W(1,1)}
$$

Form of $W(\sigma_A, \sigma_P)$: let $W(0,0)=1$ (since only ratio of weights matter)

$$
W(0,1) = \frac{[P]}{K_p}, \quad W(1,0) = \frac{[A]}{K_A}
$$

$$
W(1,1) = \omega \cdot \frac{[A]}{K_A} \cdot \frac{[P]}{K_p}
$$

$\omega = e^{-E_{\text{act}}/kT}$ ("cooperativity factor")

for $\omega[P]/K_p \ll 1$, $P = \frac{[P]}{K_p} \frac{1 + \omega[A]/K_A}{1 + [A]/K_A} \Rightarrow [m]^* = m_0 \cdot \frac{1 + \omega[A]/K_A}{1 + [A]/K_A}$

$\omega m_0 \rightarrow \ln [m]^*$ log-log slope ("sensitivity")

$\omega m_0 = \frac{\alpha[g][P]}{\beta K_p}$

$K_{a/\omega} \leftrightarrow K_A \ln([A])$

max fold change ("capacity")
sensitivity increased by, e.g., TF-TF interaction

\[
[m]^* = m_0 \cdot \frac{1 + \omega ([A]/K_A)^n}{1 + ([A]/K_A)^n}
\]

for \( \omega [P]/K_p \ll 1 \),

\[
\mathcal{P} = \frac{[P]}{K_p} \frac{1 + \omega [A]/K_A}{1 + [A]/K_A}
\]

\[
[m]^* = m_0 \cdot \frac{1 + \omega [A]/K_A}{1 + [A]/K_A}
\]

3. Repression by promoter occlusion

\[
W(\sigma_R = 1, \sigma_P = 0) = [R]/K_R,
\]

\[
W(\sigma_R = 0, \sigma_P = 1) = [P]/K_P,
\]

\[
W(\sigma_R = 1, \sigma_P = 1) = 0
\]

\[
\mathcal{P} = \frac{W(0,1) + W(1,1)}{W(0,0) + W(0,1) + W(1,0) + W(1,1)} = \frac{[P]/K_p}{1 + [P]/K_p} \approx \frac{1}{1 + [R]/K_R}
\]

\[
[p]^* = p_0 \cdot \frac{1}{1 + ([R]/K_R)^n}
\]

-- large \([R]\) can provide arbitrarily strong repression according to model
-- "promoter leakage" provides the lower limit on \([m]^*\)
-- high TF conc often generate toxic side effects
-- interaction again leads to cooperativity
4. Induction of TF

\[ X + I \xrightarrow{k_+} XI \]

- Dissociation constant:
  \[ K_i = \frac{[X][I]}{[XI]} = \frac{k_-}{k_+} \]

- Total concentrations:
  \[ [X]_{tot} = [X] + [XI] \]
  \[ [XI] = [X]_{tot} \frac{[I]}{[I] + K_i} = [X]_{tot} \frac{[I]_{tot}}{[I]_{tot} + K_i} \]

- “Activated TF” \( X^* \):
  - Form of TF able to bind specifically to DNA or able to activate RNAp
  - If \( X^* = XI \), then \( [X^*] = [X]_{tot} \frac{[I]}{[I] + K_i} \)
  - If \( X^* = X \), then \( [X^*] = [X]_{tot} \frac{K_i}{[I] + K_i} \)

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Simple circuit using transcriptional control

Consider tsx init control only (for simplicity)

\[ \frac{dp_i}{dt} = \alpha - \lambda \ [p_i] \quad \text{with} \quad \lambda = \text{growth rate}; \quad \alpha = \alpha_0 \cdot \mathcal{G} \]

\[ \mathcal{G}_A = \frac{G_{A_0}^{-1} + ([A]/K_A)^{n_A}}{1 + ([A]/K_A)^{n_A}} \]

\[ \mathcal{G}_R = \frac{1 + ([R]/K_R)^{n_R}}{1 + ([R]/K_R)^{n_R}} \]
1. Negative autoregulation
   (a very common network motif)

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

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

Steady-state solution: \([R]^* = \left( \frac{\alpha_0}{\beta_0 K_R} \right)^{1/(n+1)}\)

- General dependence of parameters on cellular physiology:
  - \(\beta_0\) = dilution due to cell growth; can vary \(\sim 10x\)
  - \(\alpha_0 > 2\)-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
  [homeostatic control]

2. Positive autoregulation

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

- 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 soln

\([A]^* = \frac{\alpha_0}{\beta_0}\]

Regime of bistability from the existence of unstable fixed point

\([A]^* = \frac{\alpha_0}{(\beta_0 f)}\)
2. Positive autoregulation

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

stability analysis (analytic):
small perturbation: \( \delta A \equiv [A] - [A]^* \)

\[
\frac{d}{dt} \delta A = \alpha_0 \frac{\partial G_A}{\partial [A]} \bigg|_{[A]^*} \cdot \delta A - \beta_0 \cdot \delta A
\]

\[
= (s^* - 1) \cdot \beta_0 \cdot \delta A
\]

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

\[ \Rightarrow s^* > 1 \] for bistability

3. Toggle switch

- qualitatively, two stable states:
  - R1 on and R2 off
  - R1 off and R1 on

- quantitatively:

\[
\frac{d}{dt}[R_1] = \alpha_1 \frac{G_{R_1}}{K_1} [R_1]^* - \beta_1[R_1]
\]

\[
\frac{d}{dt}[R_2] = \alpha_2 \frac{G_{R_2}}{K_2} \left( \frac{[R_1]^n}{K_1^n} \right)^{1/n} - \beta_2[R_2]
\]

\[
\left\{ \begin{align*}
\ln ([R_1]) & \sim \alpha_1/\beta_1 f_1 \\
\ln ([R_2]) & \sim \alpha_2/\beta_2 f_2
\end{align*} \right.
\]

\[
\left\{ \begin{align*}
\ln ([R_1]) & \sim \alpha_1/\beta_1 f_1 \\
\ln ([R_2]) & \sim \alpha_2/\beta_2 f_2
\end{align*} \right.
\]
3. Toggle switch

- bistability favored by
  - $n_1 \cdot n_2 \gg 1$
  - large $f_1$ and $f_2$
- appropriate ranges of $K, \alpha, \beta$
- defining feature of bistability: remain in a state for a “long” time after initialized to it
- initialization by changing
  - $K$ (via inducer)
  - $\alpha$ (via activator/repressor)
  - $\beta$ (via proteolysis, temperature)
- qualitatively, two stable states:
  - R1 on and R2 off
  - R1 off and R1 on
- quantitatively:

\[
\frac{d}{dt}[R_1] = \alpha_1 \cdot G_{R_1} \left( \frac{[R_1]}{K_1} \right) - \beta_1 \cdot [R_1]
\]
\[
\frac{d}{dt}[R_2] = \alpha_2 \cdot G_{R_2} \left( \frac{[R_2]}{K_2} \right) - \beta_2 \cdot [R_2]
\]
\[
\ln ([R_1]) = \alpha_1 / \beta_1
\]
\[
\ln ([R_2]) = \alpha_2 / \beta_2
\]

4. Oscillators

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

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