Life of a bacterium:

mole + energy \rightarrow \text{biomass}

**TABLE 1. Typical elemental composition of biological specimen**

<table>
<thead>
<tr>
<th>Element</th>
<th>Tissue$^a$</th>
<th>Bacteria$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>N</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>H</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>O</td>
<td>0.25</td>
<td>0.23</td>
</tr>
<tr>
<td>P + S + others</td>
<td>0.02</td>
<td>0.10$^a$</td>
</tr>
</tbody>
</table>

[Heldal et al, 1985]

- molar composition: \( \text{CH}_{1.5}\text{O}_{0.35}\text{N}_{0.24} \) (+S, P, Mg, Fe, …)
- algae (photosynthesis):
  \( \text{CO}_2 + \text{H}_2\text{O} + \text{N}_2 + \text{photons} \rightarrow \text{biomass} + \text{O}_2 \)
- \( \text{E. coli} \) (minimal medium):
  \( \text{glucose} + \text{NH}_3 \rightarrow \text{biomass} + \text{CO}_2 \)

Learning from the growth curve

[Monod, Ann Rev Microb, 1949]

- OD$_{600}$ = biomass content
  [1 OD·ml = 0.5mg CDW]
- saturation OD \( \rightarrow \) yield
- (lag: transition from pre-shift phase)

Can we predict GR & yield?

Environmental factors: nutrient types & conc, temperature, pH, osmolarity, drugs, …

Genetic factors: enzymes & regulation

\[
\frac{dM}{dt} = \lambda \cdot M
\]
growth of E. coli

Can we predict GR & yield?

environmental factors: nutrient types & conc
temperature, pH, osmolarity, drugs, …
genetic factors: enzymes & regulation

What does it take to replicate a cell?

protein = defined sequence of 20 amino acids
protein synthesis: ribosomes

What does it take to replicate a cell?
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protein synthesis: ribosomes

>85% of all RNA up to 1/3 of all proteins

condition-dependent

DNA
RNA
protein
other

charged tRNA

amino acid

mRNA

protein = defined sequence of 20 amino acids

protein synthesis: ribosomes

>85% of all RNA up to 1/3 of all proteins
protein = defined sequence of 20 amino acids

protein synthesis: ribosomes

→ amino acids & ATP from metabolic reactions

metabolism

• sequester & breakdown nutrients
  - derive energy
  - generate carbon precursors
  - sequester N, S, P, metals

biosynthesis

("precursors" to "building blocks")
  - amino acid
  - nucleic acid
  - lipids
  - co-enzyme (or ‘co-factor’)

→ but many organisms use fermentation even with oxygen (Crabtree effect); why?
metabolism
- sequester & breakdown nutrients
  - derive energy
  - generate carbon precursors
  - sequester N, S, P, metals
- biosynthesis of building blocks
  - amino acid
  - nucleic acid
  - lipids
  - co-enzymes
- degradation/recycling (e.g., mRNA)
- typical biochemical reaction:
  \[ S + Cb \rightleftharpoons Sb + C \]
  - S: substrate
  - b: component (e.g., CH₃, NH₂, e⁻)
  - C: co-enzyme
  (needed for difficult reactions)
  ➔ most reactions catalyzed by enzymes (proteins)
  ➔ flux of the products and “by-products” need to be balanced

metabolic control via coordinated regulation of enzyme abundance/activity

feedback inhibition by end-product
- 1st reaction of pathway often inhibited by product
- same enzymes used for synthesis of valine and isoleucine
- must have enzymes responding differently to different products (isozymes)
- in E. coli K-12, ilvG is defective
  ➔ valine sensitivity in minimal media
  ➔ α-ketobutyrate toxicity (repressed by isoleucine)

Also: negative feedback regulation of enzyme expression in response to end-product accumulation
1. **Gene regulation**

\[
\frac{d[R]}{dt} = \alpha_R G_R \left( \frac{[R]}{K_R} \right) - \beta_0 [R] \Rightarrow [R'] = \left( \frac{\alpha_R}{\beta_0 K_R} \right)^{1/(n_R+1)} \text{ for } \alpha_R / \beta_0 > K_R
\]

Effect on enzyme:

\[
\frac{d[E]}{dt} = \alpha_E G_E \left( \frac{[E]}{K_E} \right) - \beta_0 [E]
\]

Steady-state soln:

\[
[E^*] = K_R \cdot \left( \frac{K_E}{K_R} \right)^n \cdot \left( \frac{\alpha_E}{\beta_0 K_R} \right)^{n_E/(n_R+1)}
\]

- can have \(\alpha_R / \alpha_E \approx \text{constant} \) if the two promoters are in close proximity
- can in principle set basal enzyme conc independent of growth conditions

---

2. **Effect of the inducer (S)**

\[
[RS] = R_S = [R] \cdot \left( \frac{[S]}{K_S} \right)^n = [R] \cdot \left( \frac{[S]}{K_S} \right)^n \text{ for } [S] \ll K_S
\]

\[
[R] = R_f = [R] \cdot \frac{1}{1 + \left( \frac{[S]}{K_S} \right)^n} = [R] \cdot \left( \frac{[S]}{K_S} \right)^n \text{ for } [S] \gg K_S
\]

- if DNA binding by R requires S (e.g., R=TrpR, S=Trp, E=TrpABCDE)

Steady-state:

\[
\alpha_R \left( \frac{R_f}{K_R} \right)^{-n_R} \approx \beta_0 [R^*]
\]

\[
\Rightarrow \frac{R_f}{K_R} \approx \left( \frac{\alpha_R}{\beta_0 K_R} \right)^{1/(n_R+1)}
\]

Enzyme level:

\[
E^*([S]) = \frac{\alpha_E}{\beta_0} \left( \frac{R_f}{K_E} \right)^{-n_E} \approx \left( \frac{[S]}{K_S} \right)^{n_E/(n_R+1)} \text{ for } [S] \ll K_S
\]

- reduce the synthesis of E as S (product) level increases
• if DNA binding requires $R_f$ (e.g., LacR, TetR, …)

then $E^*(\left[S\right]) = \left[E^*\right] \frac{1 + \Omega \left(\left[S\right] / K_S\right)^m}{1 + \left(\left[S\right] / K_S\right)^n}$,

with $m = + \frac{n_S \cdot n_E}{n_R + 1}$,

$\Omega = \left(\frac{K_E}{K_R}\right)^{n_E} \left(\frac{\alpha_E}{\beta_E K_R}\right)^{\frac{n_S}{n_R \left(n_R + 1\right)}}$

Note: $m = \pm \frac{n_S \cdot n_E}{n_R + 1}$ can take on large range of values

- if $|m| \gg 1$, abrupt transition or strong buffer
- if $|m| \ll 1$, gradual control (dimmer dial)
Dependence on growth conditions:

\[ E^*([S]) = \frac{E^*}{1 + \Omega ([S]/K_s)^n} \]

\[ [E^*] = K_R^{\frac{\alpha_{E}}{\alpha_s}} \left( \frac{K_E}{K_R} \right)^{\frac{n_s}{n_s+n_s+1}} \left( \frac{\alpha_s}{\beta_{s,R}} \right)^{\frac{n_s}{n_s+n_s+1}} \]

\[ \approx K_R^{\frac{\alpha_{E}}{\alpha_s}} \text{ for } n_s = n_e \gg 1, K_R = K_E, \alpha_s \ll \alpha_E \]

\( \Rightarrow \) near independence with -ve feedback

\( \Rightarrow \) still tunable by inducer

Expt’I test:

\[ \text{P}_{\text{con}} \quad \text{tetR} \]

\[ \text{P}_{\text{tet}} \quad \text{tetR} \]

\[ \text{P}_{\text{tet}} \quad \text{lacZ} \]

3. Metabolic feedback

- regulation of E by S is often a form of feedback control

\( \Rightarrow \) include the synthesis of S by E

example: lactose transport and utilization

\( \text{LacR (encoded by } \text{lacI}) \) weakly expressed constitutively
and exerts coop strong repression of the \( \text{lacZYA} \) operon due to DNA looping

- want to inactivate LacR when lactose is present externally (and glucose absent)

- but entry of lactose requires the Lac permease (encoded by \( \text{lacY} \))
• lactose is not an inducer of LacR
• lactose is degraded by β-galactosidas (encoded by lacZ)

β-galactosidase (LacZ)

lactose (internal) → galactose + glucose

• actual inducer is allo-lactose (minor by-product of lactose degradation)
  ➔ also requires LacZ

β-galactosidase (LacZ)

lactose (internal) → allo-lactose

➔ induction of the lac operon (by allo-lactose) requires
  expression of the operon (LacY + LacZ) = positive feedback
  ➔ allo-lactose further degraded by LacZ

regulatory circuit for lactose transport/utilization:

L_{ex} → L_{in} → G → glycolysis

Y → Z → Z → A

simplified system: use lactose analogue (TMG)

• inducer of LacR
• non-hydrolyzable
• still requires LacY for entry

T_{ex} → T_{in} → dilution and/or back diffusion
simplified system: \[ \frac{d[Y]}{dt} = k_Y[Y_e] \cdot Y^*(T^-) - \lambda \cdot [T] \]

\[ Y^*(T^-) \approx Y_{\text{min}} \left( 1 + \frac{\Omega(T^-) / K_T}{1 + \left( T^- / K_T \right)^m} \right)^m \]

- TMG response is history-dependent
- to damp out short-time variation?

\[ \ln Y^* \]

• TMG response is history-dependent
• glucose effect I (“inducer exclusion”):
  -- transport of glucose reduces LacY activity (reduces \( k_Y \))
  -- same effect as reduced TMG
• glucose effect II (“catabolite repression”):
  -- transport of glucose reduces \( Y_{\text{min}} \), \( \mu \) (via CRP-cAMP)
full lac system:

\[ \frac{d[L]}{dt} = k_f \cdot [L_{ex}] \cdot Y^*([A]) - k_z \cdot [L] \cdot Z^*([A]) \]
\[ \frac{d[A]}{dt} = k_{z1} \cdot [L] \cdot Z^*([A]) - k_{z2} \cdot [A] \cdot Z^*([A]) - \lambda \cdot [A] \]
\[ Y^*([A]) = \frac{1 + \Omega ([A]/K_A)^m}{1 + ([A]/K_A)^m} \]

- include hydrolysis of substrate
- pos & neg feedback
- dilution negligible
- at steady-state: \([A] = (k_{z1}/k_{z2})[L] \sim (k_{z2}/k_{z1}) (k_Y/k_Z)[L_{ex}]\)

- no bistability; no history-dependence
- onset depends on \(k_Y\) (controlled by glucose)

Is the lac system an exception?

glycerol transport and utilization

- glp operons repressed by GlpR and activated by Crp-cAMP
- GlpF: allows glycerol influx
- GlpK: converts glycerol to g3p
- GlpD: “consumes” g3p
- g3p: inhibits GlpR
- pos + neg feedback

\[ \text{glyc} \rightarrow \text{g3p} \rightarrow \text{dhap} \rightarrow \text{glycolysis} \]

- same regulatory strategy as lac

\[ \text{glucose} \rightarrow \text{glyc} \rightarrow \text{g3p} \rightarrow \text{dhap} \rightarrow \text{glycolysis} \]
Is the lac system an exception?

Tryptophan transport & degradation

- Promoter activated by CRP-cAMP and txs attenuation (TnaC) relieved by trp
- TnaB: low affinity trp transporter
- TnaA: degrades trp into pyr, NH₄, indole
  - pos + neg feedback

Note: low affinity (high capacity) transporter is typically dominant when the ext substrate level is high; used for general catabolic purpose.

Is the lac system an exception?

Tryptophan transport & utilization

- Mtr: high affinity Trp transporter (specific for trp usage)
- repressed by TrpR
- repression of TrpR requires trp
  - negative feedback

"fetch trp as needed"

\[
\frac{d}{dt}[T] = k_{mtr} \cdot Mtr^\gamma(T) - \gamma
\]

rate of trp consumption by ribosomes
Amino acid biosynthesis: **Tryptophan biosynthesis pathway**

- **Central carbon precursors**
  - chorismate
  - indole
  - serine
  - tryptophan

- **TrpR-trp**
  - tRNA^{trp-trp}

- **ln [TrpEDCBA]**

\[
\frac{d}{dt}[\text{trp}] = k_{\text{trp}} \cdot \text{Trp}^*([\text{trp}]) - \gamma
\]

**Coordination with Mtr:**

\[
\frac{d}{dt}[\text{trp}] = k_{\text{trp}} \cdot \text{Trp}^*([\text{trp}]) - \gamma + k_{\text{Mtr}} \cdot \text{Mtr}^*([\text{trp}])
\]

- **Use Mtr if trp available in the medium**

- **ln [Mtr]**

\[
\frac{d}{dt}[\text{trp}] = k_{\text{trp}} \cdot \text{Trp}^*([\text{trp}]) - \gamma
\]

- **ln([trp])**

**的增长率 (1/hr)**

**no trp**

**w/ trp**

**更快的增长**

**更慢的增长**

- **TrpR-trp**
  - trpR
  - trpL
  - trpE
  - trpD
  - trpC
  - trpB
  - trpA

- **蛋白合成**

- **γ / k_{trp}**