Enzyme Activity: Control
Control of enzyme activity essential to maintain homeostasis

- Enzyme activities can be upregulated or downregulated
  - depends on metabolic state or needs of a cell
- Metabolic co-ordination necessary to prevent uncontrolled growth or catabolism
  - prevent conflict of opposing metabolic pathways
    - Glycogen synthesis and breakdown
    - Glycolysis and gluconeogenesis

Regulation of metabolic processes accomplished by controlling enzyme activity
Availability of substrates for MP

Regulatable enzymes in MP

Control of enzyme levels

Facilitated/Active transport

Restricted diffusion of substrates

Enzyme Synthesis

Enzyme Degradation

Interaction with Ligands

Covalent Modification

Control of enzyme activity

Compartmentation

Signal transduction
Neurotransmitters; Hormones;
Growth factors; Pheromones

Ion channels
Transmembrane receptors with intrinsic PK activity

Intracellular receptors

Membrane receptors

Second messengers

Receptors

Facilitated/Active transport

Restricted diffusion of substrates

Availability of substrates for MP

Control of enzyme levels
Enzyme activity control mechanisms

- [S] and [P]
- Allosteric Effectors
- Reversible Covalent modification
- Irreversible Proteolytic activation
- Stimulation and inhibition by control proteins
- Control of enzyme synthesis
  - Transcriptional control
  - Translational control
- Control of enzyme degradation
- Compartmentalization

} medium

Short term metabolic regulation

Long term metabolic regulation
[S] and [P]

Substrate level control

- Direct interaction of enzyme with S and P
- [S] and [P] control reaction rate
- P acts as competitive inhibitor
- Hyperbolic binding curve (noncooperative)
- Not sufficient or sensitive enough

Diagram:

1. Glucose + ATP → Heksokinase → Glucose-6-P + ADP
Feedback Control

- Control points in metabolic pathways
  - Regulation of rate-limiting enzyme(s) early in MP
  - Conserve energy and metabolites
    - AcetylCoA inhibits pyruvate kinase
    - Control of purine/pyrimidine biosynthesis
Mammalian Hexokinase
Katzen and Schimke (1965), purified four forms of hexokinase in mammalian tissues based on their different charges, and by their kinetics and stability properties.
Evolution and Structure

Insensible (yeast HK, glucokinase) or sensible (starfish HK) to G-6-P

Gene duplication + fusion

50 KDa

100 KDa

Mammalian hexokinase I, II, III
Sensible to Glc-6-P

Glc / Glc-6-P - Pi

Glc - ATP / Glc-6-P - P_i

Catalytic domain

Regulatory domain

Conserved open twisted β-sheet motif
Structure of Mammalian Hexokinase I

- Open and closed conformation. Each domain has a large and small lobe that form a cleft where the substrates (Glc / ATP) and the effectors (Glc-6-P / P_i) bind.

  - **Regulatory domain**: closed conformation is induced by Glc binding.
  - **Catalytic domain**: ATP or Glc-6-P must also bind to the active site to induce the closed conformation.

- Contacts between the two domains. Transitional α-Helix and two loops that would be involved in allosteric regulation.
Hexokinase I catalyzes the phosphorylation of Glc in the 6-OH position. High levels of the product inhibit HKI, but this inhibition is reversed by Pi.

If hexokinase I were not inhibited by its product, as a consequence of its low Km (100 fold lower than the normal [Glc]), it would phosphorylate large amounts of Glc, reducing the level of Pi available to generate ATP. If Po$_2$ is low, NADH +H$^+$ accumulates, TCA cycle shuts down, and the only source of ATP would be glycolysis; in order to provide enough energy for the brain, more Glc has to be phosphorylated. Under these conditions, Pi accumulates (no Oxid. Phosphorylation), and it counteracts the inhibitory effect of Glc-6-P.
Regulatory mechanism

- **Glc-6-P binds to ATP, not Glc, binding site** (vestigial ATP binding site at the regulatory domain).
- In the regulatory domain, $P_i$ binds with high affinity to the same site that the phosphoryl group of Glc-6-P, and with a low affinity to the catalytic domain.

### Competitive inhibition

Glc-6-P binds to the C-terminal domain stabilizing the closed conformation and competing directly with ATP for the active site; in this model, ATP mediates the antagonism between $P_i$ and Glc-6-P.

### Allosteric mechanism

Glc-6-P binds to the N-terminal domain generating the closed conformation, which further stabilizes the flexible subdomain of the C-terminal half and prevents ATP from completely bind to the C-terminal half. In this case, $P_i$ directly competes for the binding site with Glc-6-P.
Regulation of HK /GK and Glc-6-Pase

Relationship between HK and GK

**Hexokinase: (Muscle)**
- High specificity for sugars
- $K_m \approx 0.1 \text{ mM} \ (<< \ 4.4 \text{ mM normal [Glc]})$
- Operates at $V_{max}$ (saturated with Glc)
- Cannot respond to small $\Delta [\text{Glc}]$.
- Inhibited by its product Glc-6-P

**Glucokinase: (Liver)**
- Lower specificity for Glc
- $K_m \approx 10 \text{ mM} \ (>> \ 4.4 \text{ mM normal [Glc]})$
- Operates below $V_{max}$ (not saturated with Glc)
- Can respond to small $\Delta [\text{Glc}]$ to keep [Glc] in blood constant.
- Not inhibited by its product Glc-6-P

Substrate level control-
hyperbolic binding
Glucose-6-phosphatase:
- Present only in liver, not in muscles.
- $K_m$ (G-6-P) $\gg [G-6-P]$ in blood.
- Not inhibited by its product Glc.
- Substrate level control
- Synthesis stimulated by glucagon.
- Committed step early in pathway, to inhibit glycolysis in liver.
- Role: to convert G-6-P obtained by glycogenolysis to Glc that can be transported to blood or brain.
Allosteric Effectors

- Allosteric enzymes
  - multisubunit proteins with multiple catalytic sites.
  - interactions between the subunits can be influenced by
    - binding of the substrate (homoallostery)
    - binding of effectors on regulatory sites (heteroallostery).

- Action of effectors
  - Alter affinity of E for its substrate
  - Change catalytic activity
    - Change in tertiary and quaternary structure of allosteric protein upon effector binding

- Rate-limiting enzymes subject to allosteric regulation
Biphasic Effect

- In addition to allosteric inhibitors (which bind to binding sites distinct from the catalytic site), allosteric enzymes may be influenced by Competitive inhibitors.

- Competitive inhibitors are substrate analogues that binds to the same catalytic site as the S.

- Low [I] would increase the ability of the E to bind S and thus increase reaction velocity.

- High [I] would block S binding in the usual way.
The Sigmoid Plot and the Hill Equation

- Non-allosteric enzyme
  - Hyperbolic plot
  - MM Kinetics

- Allosteric enzyme
  - Sigmoidal plot
  - Non MM kinetics

- $h = \text{Hill coefficient}$
  - Value gives a measure of cooperativity

- $h = 1$: No cooperativity, graph is hyperbolic
- $h > 1$: + Cooperativity, sigmoidal curve
- $h < 1$: - Cooperativity, sigmoidal curve

\[
V_0 = \frac{V_{\text{max}} [S]}{K_m + [S]}
\]

\[
V_0 = \frac{V_{\text{max}} [S]^h}{K^{h_{0.5}} + [S]^h}
\]
Advantages of cooperativity
Allosteric Regulation of Aspartate Carbamoyltransferase (ATCase)

Control points in pyrimidine synthesis
Formation of N-carbamoyl-L-aspartate from carbamoyl phosphate and aspartate is the first committed step in a series of reactions that lead to synthesis of pyrimidine nucleotides. Control at or near this point is essential. In prokaryotes, the ATCase is regulated; in most eukaryotes, regulation is on the carbamoyl synthetase II.

Regulation of ATCase: Allosteric effectors
CTP = inhibitor: If [CTP] is high, cell does not require more pyrimidines.
ATP = activator: If [ATP] is high, cell is in purine-rich state and needs more pyrimidines.
Also, high [ATP] means energy-rich cell conditions exist under which DNA and RNA synthesis will be active.
Quaternary structure
12 subunits
 6 catalytic (3 x 2)
    Asp & Carbamoyl phosphate
 6 regulatory (2 x 3)
    ATP (activator) $\rightarrow$ R state
    Lower [S] required to reach $v$
    CTP (feedback inhibitor) $\rightarrow$ T-state
    Higher [S] required to reach $v$
Cumulative covalent modification by Adenylation

AT = adenylyl transferase

$\text{P}_{II} = \text{regulatory protein;}$

GS = glutamine synthetase;

UT = uridylyl transferase
Regulatory Mechanisms
Covalent Modification
Reversible

- Enzyme is inactive (on standby) until activated by covalent modification
- Associated with regulatory cascades in which the original signal is amplified

Generalized scheme for reversible covalent modification reactions. A generic group $X$ is transferred to or removed from a specific acceptor residue $A$ on the protein.
Common Reversible Covalent Modifications
# Major targets of covalent modifications

<table>
<thead>
<tr>
<th>Modification</th>
<th>Target site</th>
<th>Example of process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>Lys, N-terminal-NH₂</td>
<td>Gene expression</td>
</tr>
<tr>
<td>ADP-ribosylation</td>
<td>Arg, Cys, Asn, Glu, Lys</td>
<td>Toxin, protein assembly</td>
</tr>
<tr>
<td>Amidation</td>
<td>C-terminal (Gly)</td>
<td>Bioactive peptides</td>
</tr>
<tr>
<td>Hydroxylation</td>
<td>Pro, Lys, Asn, Asp</td>
<td>Collagen structure</td>
</tr>
<tr>
<td>Methylation</td>
<td>Arg, Lys, His, Glu, isoAsp</td>
<td>Protein repair, chemotaxis</td>
</tr>
<tr>
<td>Myristoylation</td>
<td>N-terminal (Gly)</td>
<td>Membrane association ?</td>
</tr>
<tr>
<td>Palmitoylation</td>
<td>Cys</td>
<td>Membrane association</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>Ser, Thr, Tyr</td>
<td>Signalling, enzyme activity</td>
</tr>
<tr>
<td>Prenylation</td>
<td>Cys</td>
<td>Signalling, oncogenesis</td>
</tr>
<tr>
<td>Sulfation</td>
<td>Tyr</td>
<td>Prot-prot interactions</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>Lys</td>
<td>Prot degradation</td>
</tr>
</tbody>
</table>
**Phosphorylation/Dephosphorylation**

- **Most common and important**
  - Implicated in virtually every process involved in cellular recognition

- **Phosphorylation**
  - **Kinase**
  - $E-OH + ATP \Leftrightarrow E-O-PO_3^{2-} + ADP + H^+$

- **Dephosphorylation**
  - **Phosphatase**
  - $E-O-PO_3^{2-} + H_2O \Leftrightarrow E-OH + HO-PO_3^{2-}$

- **Specific residues with -OH groups**
  - Ser, Thr, Tyr
Activation by Phosphorylation: Glycogen Phosphorylase

Regulatory cascade controlling glycogen breakdown in muscle cells after epinephrine stimulation ultimately leads to activation of glycogen phosphorylase by covalent modification (phosphorylation)
Superposition of control mechanisms

Covalent modification
Phosphorylation of Ser 14

Allosteric Regulation

\[ \uparrow \text{AMP and Ca}^{2+}, \quad \downarrow \text{ATP and high [Glucose]} \]

If [Glc] is very low, the allosteric inhibition of Glc is abolished, and phosphorylase b (R-state) can also catalyse release of Glc-1-P from glycogen

Hormonal regulation

During stress: Epinephrine (muscles) or Glucagon (liver) initiates a regulatory cascade that activates phosphorylase kinase: more Glc-1-P is released

When [Glc] is high, insulin activates a regulatory cascade that activates phosphatase, and Glc-1-P release decreases.
Proteolytic Activation

Irreversible

Zymogens = inactive precursor of enzyme
Cleavage of peptide bond → active enzyme
Zymogen activation:
  Digestive enzymes
  Blood-clotting Factors
  Hormones

Upon proteolytic cleavage, the zymogen undergoes a conformational change resulting in the active enzyme E with its active site exposed. S can now access the catalytic site.

Irreversible proteolytic inhibition may also occur owing to the binding of specific protease inhibitors
Proteolytic Cleavage of Pancreatic Proteases

Zymogens are synthesised in pancreas and secreted through the pancreatic duct into the duodenum in response to a hormonal signal (somatotropin). They are cleaved in the duodenum to yield the active enzymes.

Trypsin autocatalyze its own activation and activate other zymogens.

**Activation of chymotrypsinogen**
Trypsin cleaves between Arg 15 and Ile 16 to form π-chymotrypsin.
Further cleavage removes residues 14-15 and 146-148 to form α-chymotrypsin.
The newly generated N-terminal peptides remain attached via the disulfide bridges.
Proteolytic activation is irreversible

- Mechanisms must exist to turn off the activating enzyme
- Irreversible binding of specific inhibitory Control Proteins
  - Protease inhibitors
  - Tight binding to enzyme active site to form stable, inhibited complexes
- Production of these inhibitors are under distinct regulatory control
  - Gene expression

Secretory pancreatic trypsin inhibitor:

- Trypsin activation can be autocatalytic.
- The pancreas protects itself by synthesizing secretory pancreatic trypsin inhibitor.
- This is a competitive inhibitor of trypsin that binds very tightly to the active site of trypsin. (This is the strongest noncovalent association known).
- Activation of pancreatic complex in pancreas is prevented by very low [SPTI].
- Zymogen activation in pancreas will lead to digestion of pancreatic tissue (acute pancreatitis) e.g. if pancreatic duct is blocked.
Control of Enzyme Degradation

Lysosomal hydrolytic enzymes
   Proteases sequestered
   Activity in acidic environment
      Turnover of long-lived proteins
      Degradation of extracellular proteins taken up by cell

Proteases in cytosol and other compartments
   Selective protein turnover related to metabolic regulation
   Protease activity must be tightly regulated
      Ubiquitin-specific proteases
      Proteasomes (giant complexes of ATP-dependent proteases)

Chemical signals for turnover
   Ubiquitination
      Oxidation of specific aa residues (metal-catalyzed)
      PEST sequences
      Particular N-terminal residues
Ubiquitination

Ubiquitin: 76 aa protein found in all eukaryotic cells
Ubiquitin COO− group is activated by thiol coupling to an activating enzyme (requires ATP)
Ubiquitin moieties are then transferred to a second enzyme, which attaches them to e-amino groups of Lys on the fated protein
Protein marked with ubiquitin is digested by ubiquitin-specific proteases in the cytosol
Proteasome destruction of proteins in cytosol

- Multicatalytic proteinase complex (MPC) (700 kDa) can either degrade some proteins directly or participate in the ubiquitin pathway.

\[ \text{ATP} \]

- Damaged proteins
- Ubiquitin-proteins

\[ \text{Oligopeptides} \]

\[ \text{Endopeptidases} \]
\[ \text{Exopeptidases} \]

\[ \text{Amino Acids} \]
Compartmentalization

**Within cells**
- **Division of labor**
  - Compartments perform specialized functions
    - Mitochondria: energy production
    - Ribosomes: protein synthesis
  - Increased efficiency of cell function
- **Regulatory function**
  - Intermediates of a pathway remain trapped inside organelle
  - Flux through pathway controlled by the rate at which carriers in membrane import substrates
  - Juxtaposition of enzymes that catalyze sequential reactions restrict diffusion of intermediates, keeping local concentrations high
    - Enzymes bound to one another in membrane
      - Enzymes of mitochondrial electron transport
    - Organized multiprotein complexes
      - Pyruvate dehydrogenase complex
    - Organized structures of associated enzymes in cytosol
      - Cytomatrix
Regulatory function....

- Regulate enzymes that require a certain environment
  - Lysosomal hydrolytic enzymes that function at acidic pH
  - Acidic environment renders macromolecules susceptible to degradation and recycling
- Protects a cell from degradation by proteases of lysosomal enzymes

Within tissues/organs

Division of labor

- Expression of proteins is tightly controlled to maximize efficiency while conserving energy and preventing toxic build-up of metabolic byproducts
  - Tryptophan hydroxylase (TPH)
    - Rate-limiting enzyme in biosynthesis of serotonin (neurotransmitter)
    - Expressed in cells of nervous system, pineal gland and digestive tract
    - Byproducts of serotonin biosynthesis is toxic
    - Adequate levels of serotonin required, so body limits those cells that produce TPH
At any given time there are thousands of different metabolic processes that occur within a cell. We have reviewed some of the mechanisms cells utilize to regulate the activity of the enzymes that are driving these reactions. Depending upon needs, a cell can stimulate either synthesis or degradation of its intracellular enzymes. Additionally, enzymatic activities can be controlled by the [S] and [P]. Other mechanisms, such as allosteric control, reversible covalent modification and proteolytic activation or compartmentalization of proteins, allow the cell to efficiently modulate the overall metabolic processes of an individual cell. These processes can operate independently or in a synergystic manner. Main objective: maintaining a well-balanced, homeostatic state.