Living cells contain thousands of metabolites linked to one another by a dizzying array of chemical reactions. These reactions link one metabolite to another and collectively are arranged into metabolic pathways, which crisscross and intersect to form a large interconnected network. Each reaction is catalyzed by one or more enzymes and many of these enzymes play a large role in controlling the flow of material through the network. In this lecture we will focus on some of the strategies used to regulate enzyme activity, and consequently, metabolic processes.
Introduction

- Metabolism comprises a vast network of interconnecting metabolic pathways.
Introduction

✦ One of the primary strategies for regulating metabolism is to regulate the activity of some of the key enzymes in this network.

✦ There are several mechanisms used to do this:
  • Allosteric Control
  • Multiple Forms of Enzymes (Isozymes)
  • Reversible Covalent Modifications
  • Proteolytic Activation
  • Controlling the level of Enzyme Present
Introduction

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Regulation by Covalent Modification

- Some enzymes are regulated by reversible, covalent modifications

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<thead>
<tr>
<th>Modification</th>
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<th>Protein function</th>
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Regulation by Covalent Modification

- Phosphorylation/Deposphorylation is the most common form of covalent modification.
  - The hydroxyl groups of Serines and Tyrosines are phosphorylated by **protein kinases** to produce phosphate esters.
Regulation by Covalent Modification

- Protein phosphatases reverse this modification.

\[
\text{Phosphorylated protein} + \text{H}_2\text{O} \xrightarrow{\text{Protein phosphatase}} \text{Orthophosphate (P}_i\text{)}
\]
Regulation by Covalent Modification

- Both phosphorylation and dephosphorylation are favorable reactions.

\[
\begin{align*}
\text{Protein-OH} + \text{ATP} & \quad \text{Protein-OPO}_3^{2-} + \text{ADP} \\
\text{Protein-OH} + \text{HOPO}_3^{2-} & \quad \text{H}_2\text{O}
\end{align*}
\]
Regulation by Covalent Modification

✦ The Protein kinases respond to different signals.

<table>
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<th>Signal</th>
<th>Enzyme</th>
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<tr>
<td>Cyclic nucleotides</td>
<td>Cyclic AMP-dependent protein kinase</td>
</tr>
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<td></td>
<td>Cyclic GMP-dependent protein kinase</td>
</tr>
<tr>
<td>Ca$^{2+}$ and calmodulin</td>
<td>Ca$^{2+}$-calmodulin protein kinase</td>
</tr>
<tr>
<td></td>
<td>Phosphorylase kinase or glycogen synthase kinase 2</td>
</tr>
<tr>
<td>AMP</td>
<td>AMP-activated kinase</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>Metabolic Intermediates and</td>
<td>Many target-specific enzymes, such as pyruvate</td>
</tr>
<tr>
<td>other “local” effectors</td>
<td>dehydrogenase kinase and branched-chain</td>
</tr>
<tr>
<td></td>
<td>ketoacid dehydrogenase kinase</td>
</tr>
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</table>

Source: After D. Fell, *Understanding the Control of Metabolism* (Portland Press, 1997), Table 7.2.
Tyrosines can also be phosphorylated
- Only observed in multicellular eukaryotes
- Tyrosine kinases are involved in growth regulation.
- Some cancers are associated with malfunctioning tyrosine kinases
Regulation by Covalent Modification

- Phosphate groups are well suited to altering an enzyme's activity.
  - Phosphorylation adds two negative charges to a protein.
  - Phosphates are effective at forming hydrogen bonds.
  - Phosphorylation provides a source of free energy for conformational changes in a protein ($\Delta G^\circ' = -50 \text{ kJ/mol}$).
  - Using enzymes to regulate enzymes can be used to produce a large amplification of a regulatory signal.
  - By using ATP as a source of phosphate groups, phosphorylation is sensitive to the cell's energy supply.
Regulation by Covalent Modification

- Both phosphorylation and dephosphorylation are favorable reactions.

```
Protein-OH + ATP → Protein-OPO_3^{2-} + ADP
Protein-OH + HOPO_3^{2-} → Protein-OH + H_2O
```

Free energy
Regulation by Covalent Modification

- The 500 or so protein kinases vary in specificity.
  - Some are specific and some are multifunctional
  - The consensus sequence for multifunctional kinases is

\[-\text{Arg-Arg-}X\text{-Ser-Z-}\]
\[-\text{Arg-Arg-}X\text{-Thr-Z-}\]

- Where $X$ is a small amino acid, viz. Gly or Ala and $Z$ is a large hydrophobic amino acid, viz. Met or Ile
Regulation by Covalent Modification

* As the protein kinases modify the activity of key enzymes, they must be regulated in response to their corresponding signal.

* **Protein Kinase A (PKA)** provides a good example.
Regulation by Covalent Modification

- **Protein Kinase A (PKA)** is involved in the “flight or fight” response.
  - This response is triggered by the release of the hormone epinephrine (adrenalin) by the adrenal glands.
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- Cyclic-AMP (cAMP) is produced as a “second messenger” in response to epinephrine.
- Cyclic-AMP (cAMP) binds to, and alters, the quaternary structure of PKA.
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Regulation by Proteolytic Cleavage

* **Proteolytic Cleavage** is used to regulate enzymes that need to be synthesized in an inactive form in one location, then transported to a different time or location, where they become active.

- Digestive enzymes
- Blood clotting proteins
- Protein Hormones (not an enzyme)
Regulation by Proteolytic Cleavage

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  - Digestive enzymes
  - Blood clotting proteins
  - Protein Hormones (not an enzyme)
Regulation by Proteolytic Cleavage

- Digestive enzymes are synthesized in an inactive form called a zymogen.

<table>
<thead>
<tr>
<th>Site of synthesis</th>
<th>Zymogen</th>
<th>Active enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Pepsinogen</td>
<td>Pepsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Chymotrypsinogen</td>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Trypsinogen</td>
<td>Trypsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Procarboxypeptidase</td>
<td>Carboxypeptidase</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Proelastase</td>
<td>Elastase</td>
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Regulation by Proteolytic Cleavage

- Chymotrypsin provides a good example.
  - Chymotrypsin is synthesized by the pancreas in an inactive form, chymotrypsinogen.
Regulation by Proteolytic Cleavage

- Chymotrypsinogen is transported to the small intestine, where it becomes activated to chymotrypsin

![Diagram showing the activation process of chymotrypsinogen](image)
Regulation by Proteolytic Cleavage
Regulation by Proteolytic Cleavage

- Digestive enzymes
  - Other examples, including other pancreatic zymogens trypsinogen, proelastase, procarboxypeptidase and prolipase, are activated by proteolytics cleavage
Regulation by Proteolytic Cleavage

- The proteolytic activation is irreversible, therefore other means must be used to inhibit the digestive enzyme.

- Protease Inhibitors
Other Serine Proteases

- Other Serine Proteases Homologues include **trypsin** and **elastase**
Regulation by Proteolytic Cleavage

- The proteolytic activation is irreversible, therefore other means must be used to inhibit the digestive enzyme.
  - Protease Inhibitors

\( \alpha_1 \)-antitrypsin
Regulation by Proteolytic Cleavage

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  - Protease Inhibitors

\[ \text{α}_{1}\text{-antitrypsin} \]
Next up

- Unit IV, Lecture 7 - Carbohydrates (Chapter 11)