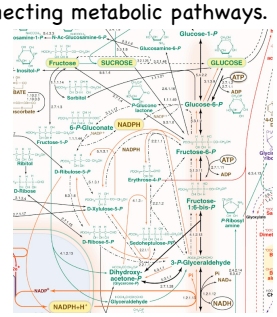
Chem 452 - Lecture 6
Regulatory Strategies
111104

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**

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Regulation by Covalent Modification

- Some enzymes are regulated by reversible, covalent modifications

Table 10.1 Common covalent modifications of protein activity

Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP ribosylation	NAD^+	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3'-Phosphoadenosine-5'-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle

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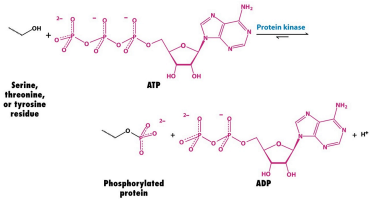
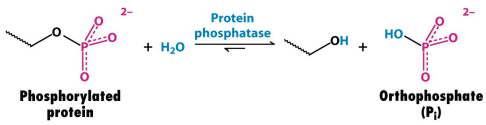
Regulation by Covalent Modification

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



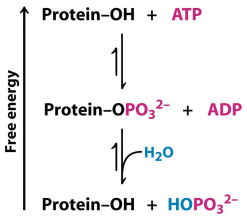
Regulation by Covalent Modification

- **Protein phosphatases** reverse this modification.



Regulation by Covalent Modification

- Both phosphorylation and dephosphorylation are favorable reactions.



Regulation by Covalent Modification

- The Protein kinases respond to different signals.

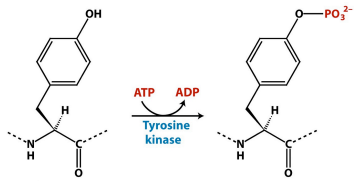
TABLE 10.2 Examples of serine and threonine kinases and their activating signals

Signal	Enzyme
Cyclic nucleotides	Cyclic AMP-dependent protein kinase
	Cyclic GMP-dependent protein kinase
Ca^{2+} and calmodulin	Ca^{2+}–calmodulin protein kinase
	Phosphorylase kinase or glycogen synthase kinase 2
AMP	AMP-activated kinase
Diacylglycerol	Protein kinase C
Metabolic Intermediates and other "local" effectors	Many target-specific enzymes, such as pyruvate dehydrogenase kinase and branched-chain ketoacid dehydrogenase kinase

Source: After D. Fell, *Understanding the Control of Metabolism* (Portland Press, 1997), Table 7.2.

Regulation by Covalent Modification

- Tyrosines can also be phosphorylated
  - Only observed in muticellular eukaryotes
  - Tyrosine kinases are involved in growth regulation.
  - Some cancers are associated with malfunctioning tyrosine kinases

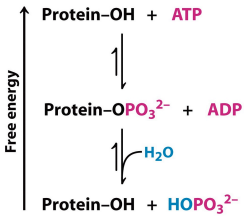


Regulation by Covalent Modification

- 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 proteins ($\Delta G^{\circ\prime}$=-50 kJ/mol)
  - Using enzymes to regulate enzymes can be used to produced 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.



Regulation by Covalent Modification

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

-Arg-Arg-X-**Ser**-Z-

or

-Arg-Arg-X-**Thr**-Z-

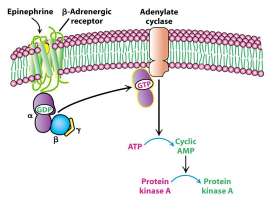
  - Where X is a small amino acid, viz. Gly or Ala and Z is a large hydrophobic amino acid, viz. Met or Ile

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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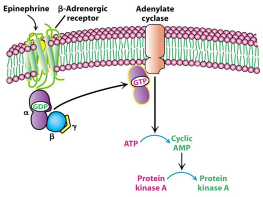
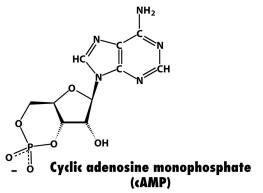
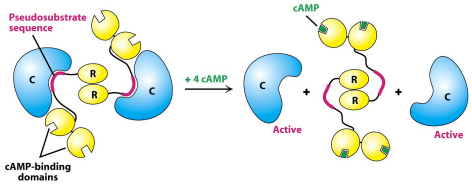




Cyclic adenosine monophosphate (cAMP)

Regulation by Covalent Modification

- 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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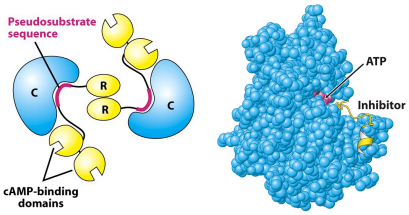

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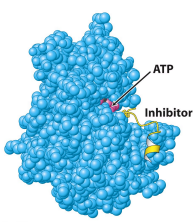

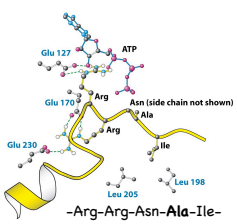

-Arg-Arg-Asn-**Ala**-Ile-

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)

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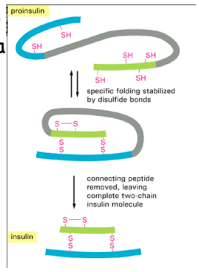


Regulation by Proteolytic Cleavage

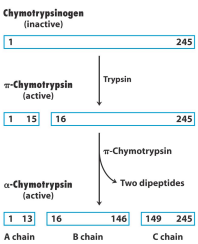
- Digestive enzymes are synthesized in an inactive form called a **zymogen**.

TABLE 10.3 Gastric and pancreatic zymogens

Site of synthesis	Zymogen	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelastase	Elastase

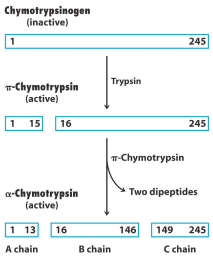
Regulation by Proteolytic Cleavage

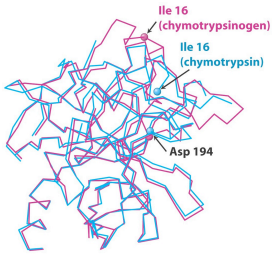
- Chymotrypsin provides a good example.
  - Chymotrypsin is synthesized by the pancreas in an inactive form, chymotrypsinogen.



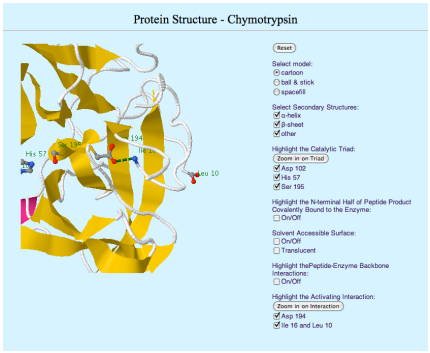
Regulation by Proteolytic Cleavage

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



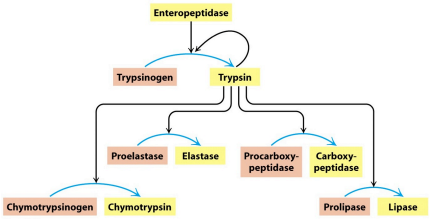


Regulation by Proteolytic Cleavage



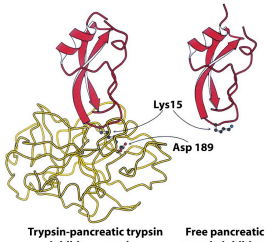
Regulation by Proteolytic Cleavage

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



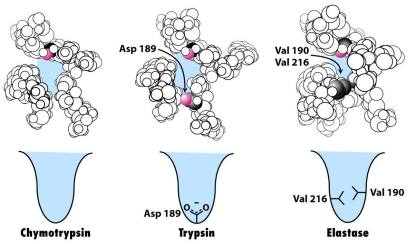
Regulation by Proteolytic Cleavage

- 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**



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α_1-antitrypsin

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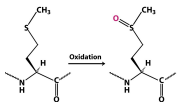

α_1-antitrypsin

Next up

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