

Overview of metabolism

Regulation of metabolism and enzymes

There are 5 principle means of regulating enzyme activity and thus metabolism

- Reversible effectors - both allosteric activation and inhibition and simple inhibition (Michealis-Menten)
- Regulation by a second protein via protein protein interactions
- Reversible covalent modification - phosphorylation - dephosphorylation, lipid modification. This method is important because it does not alter the total amount of protein and it is easily reversed depending on cellular needs
- Zymogen / proteolytic activation - this is an irreversible mechanism and must have tight control for the activation.
 - most digestive enzymes such as trypsin and chymotrypsin
 - blood clotting proteins are also commonly activated by this means.

Why is this important?

Irreversible activation by cleavage of one or more peptide bonds.

Usually protein is made in one organ and secreted in the inactive form and then made active at a distal site/tissue

- some hormones (insulin)
- digestive enzymes

- Availability - there are several means by which the cell controls metabolism this way.
 - altering the physical location of the enzyme with or away from the substrate obviously controls the activity. Translocation of proteins from one organelle to another is the mode of operation.
 - Sequestering or controlling the enzyme from it's substrate (glucose-6 phosphate is in the cytosol whereas the enzyme glucose 6 phosphatase is in the inside of the endoplasmic reticulum. The substrate is transported across the ER membrane when the reaction is needed)
 - Turnover - proteins generally have a defined half-life in the cell. Proteins are regularly being made and degraded. Altering either of these processes changes the total concentration of enzyme in the cell available for metabolism. The genetic control or rate of protein expression will play an important role in this regulation.
 - Various pathways can be differentially regulated by the use of Isozymes - Enzymes that catalyze the same reaction but are different kinetic properties and regulation

And now a few methods in detail:

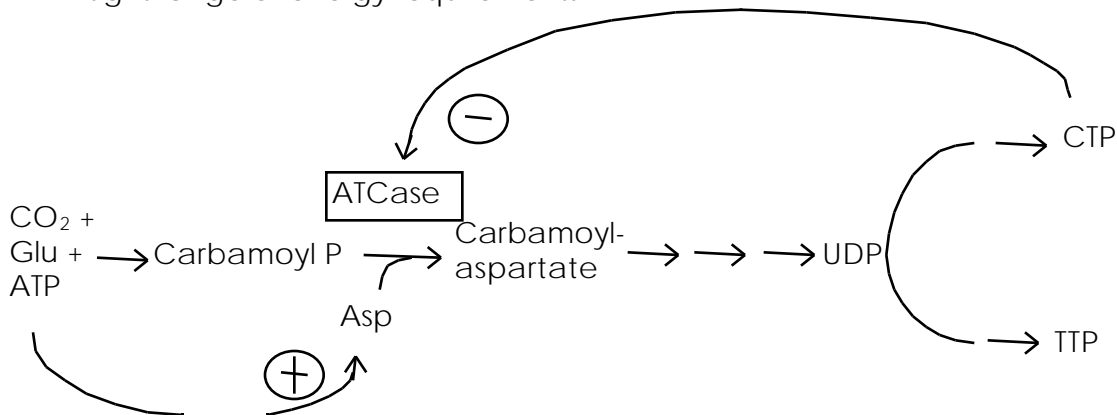
Allosteric activation/inhibition

- In most metabolic pathways there is at least 1 key enzyme
- Usually these pacemaker enzymes are in the first committed step in the pathway.
- Many times regulated by both feed forward and feedback mechanisms
- Allosteric alterations allow the enzyme to respond to changes in intracellular conditions

- If the allosteric modifying ligands influence the binding of an identical ligand, the effects are homotropic
- If the binding of a ligand affects the binding of a different ligand then the allosterism is heterotropic. This is also called cooperatively

Aspartate Transcarbamoylase (ATCase)

- Involved in denovo synthesis of nucleotides (CTP and TTP)
- Sigmoidal kinetics
 - enzyme activity tightly controlled, indicator of pacemaker enzyme
 - positive cooperativity encourages the binding to occur over a narrow range of substrate
 - tight range of energy requirements



- Enzyme under tight control of aspartate
- High concentrations of substrate or product allosterically affect enzyme kinetics
- Feed forward activation can overcome feed back inhibition

Covalent modification - Protein kinases

Phosphorylation/dephosphorylation

- Most common method of reversible modification
 - activation and localization
- Up one-third of all cellular proteins are phosphorylated (so since there are ~30,000 genes, that would be ~10,000 phosphoproteins/organism, and perhaps a third of that in any given cell type, not including alternatively spliced isoforms).
- Leads to a very fast response to cellular stress, hormonal changes, learning processes transcription regulation
- Different than allosteric or Michealis Menten regulation
- Phosphorylation stabilized thermodynamically
 - only half-available energy used in adding phosphoryl to protein
 - change in free energy forces phosphorylation reaction in one direction
- Phosphatases reverse direction
- The rate of reaction of most phosphatases are 1000 times faster
- Phosphorylation occurs on serine threonine or tyrosine residues
- What differences occur due to the addition of a phosphoryl group?

- Regulation of protein phosphorylation varies depending on protein
 - some turned on or off
 - most kinases are regulated
 - phosphatases generally not regulated
 - can lead to large amplification of original signal
- Four general classes of protein kinases, based on substrate (both sequence and amino acid phosphorylated), homology and regulation mechanisms (thousands of kinases)

Protein Kinase A (PKA) pp 676

- Activated by cyclic Adenosine Monophosphate (c-AMP)
- Recognizes specific sequences in substrate
 - Arg-Arg- X - Ser/Thr - Z
 - X = small aa, Z = hydrophobic aa (not Tyr)
- Called consensus sequence
- Important in regulation by hormones and neurotransmitters
- c-AMP produced from ATP by adenylyl cyclase
- PKA is a heterotetramer, not linked together by peptide bond
- Regulatory subunits - Arg-Arg- **Gly** - Ala - Ile
- Pseudosubstrate - binds deep in cleft between catalytic subunits
- Competitive inhibitor at active site
- Binding of c-AMP to R subunits shifts Pseudosubstrate away from active site
- Catalytic subunits now active
- Degradation of c-AMP to AMP by another enzyme leads to removal of c-AMP from R subunits and reformation of inactive heterotetramer

Protein Kinase C (PKC) pp 684 and 685

- Ser/Thr protein kinase
- Monomer - pseudosubstrate part of whole protein (polypeptide)
- Activated by increases in cellular Ca^{++} and the lipid diacylglycerol (DAG) - DAG is also called a tumor promoter....
- DAG made by other enzymes in response to hormonal changes.
 - Very transient molecule, often use phorbol esters to study
- No real stringent consensus sequence - usually Arg rich targets
- Over 23 isoforms based in three categories
 - conventional PKC - Ca^{++} and Lipid regulated
 - novel PKC - only Ca^{++} activated
 - Atypical PKC - not regulated by either Ca^{++} or lipid
- Forms are generally splice variants (alterations at the gene level)
- inactive in resting state bound to pseudosubstrate, found in cytosol
- Activated after ATP, Ca^{++} and DAG
- translocation to membrane (why?)

Protein Tyrosine Kinases (PTK) pp 677

- Phosphorylates at a tyrosine residue only
- Several kinds of cancer are mutated versions of tyrosine kinases
- 2 classes; receptor or cytosolic
- Receptor tyrosine kinases
 - receptor of hormones/growth factors

- found on both sides of the cell membrane
- extracellular portion binds hormone and alters conformation through the membrane and the cytosolic portion
- now the kinase part of the receptor is active
- Cytosolic or non-receptor
- Part of the Src family -mutated form originally found in rous sarcoma virus
- Usually regulated by other tyrosine kinases (receptor kinases)
- only a handful of this kind
- have the same catalytic domains
- found nearly in all cells
- Murine lymphoma (leukemia) formed when tyrosine kinase "abl" is uncontrolled - turned on and can't be inhibited. Sends out a signal to the cell as if growth factor was always there. Results in continual cellular growth and tumor formation

Protein Kinase B (PKB)

- Newer type of kinase - not much known also known as AKT
- Most regulated by proteins which bind GTP, similar to Ras
- This enzyme has a PH domain - **p**leckstrin **h**omology
- lipid binding (inositol lipids, NOT DAG)
- Growth factor activated
- First found from thyroid tumor
- Only two or so targets known
- Seems to be involved in insulin activation of cellular functions