Enzyme Regulation - What Factors Influence Enzymatic Activity?

Principle means of regulating enzyme activity
- Reversible, non-covalent (allosteric and simple-MM) – typically small molecules
- Reversible, covalent
- Protein-Protein interactions
- Zymogen activation
- Protein expression and degradation
- Availability (both of enzyme and substrate)

**Reversible Noncovalent**
Simple activation and inhibition by small molecules – substrate, natural regulators of enzymes

MM kinetics $K_m$, $V_{max}$ – competitive, non competitive…

Substrate inhibition or activation

The availability of substrates and cofactors usually determines how fast the reaction goes
As product accumulates, the apparent rate of the enzymatic reaction will decrease

**Reversible Noncovalent: Allosteric**
Action at “another site”
Enzymes situated at key steps in metabolic pathways are modulated by allosteric effectors
These effectors are usually produced elsewhere in the pathway
Effectors may be feed-forward activators or feedback inhibitors
Kinetics are sigmoid (“S-shaped”)

**Reversible Covalent**
Examples include: 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

**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 ....
Tony Hunter (Salk Institute) ‘87 predicted 1001 protein kinases - more like 2000, but what is 999 proteins among friends?

Over-expression and constitutive activation of protein kinases exist in many human cancers; >80% of the proto-oncogenes and oncogenes identified encode protein tyrosine kinases.
What Kinds of Covalent Modification Regulate the Activity of Enzymes?

Protein kinases phosphorylate Ser, Thr, and Tyr residues in target proteins.

Kinases typically recognize specific amino acid sequences in their targets.

In spite of this specificity, all kinases share a common catalytic mechanism based on a conserved core kinase domain of about 260 residues.

Kinases are often regulated by intrasteric control, in which a regulatory subunit (or domain) has a pseudosubstrate sequence that mimics the target sequence, minus the phosphorylatable residue.

Covalent modification - Protein kinases

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)

• Activated by cyclic Adenosine Monophosphate (cAMP)
• 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
• cAMP produced from ATP by adenylyl cyclase
• PKA is a heterotetramer, not liked 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 cAMP to R subunits shifts Pseudosubstrate away from active site
• Catalytic subunits now active
• Degradation of cAMP to AMP by another enzyme leads to removal of cAMP from R subunits and reformation of inactive heterotetramer
Protein Kinase C (PKC)

- 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)

- 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
**Protein-Protein Interaction**

Based on interface between two proteins
- can cause allosteric regulation but this style of interaction is different than between enzyme subunits

Protein binding can alter structure of second protein – one of the pairs are often regulated by a small molecule or covalent regulation

GTP Binding Proteins as an example

**Zymogen Activation**

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

**Protein Concentration**

Protein lifetime in cells is not indefinite
- Proteases – regulated activity within cells
- Proteins are usually **tagged for selective** destruction in proteolytic complexes called **proteasomes** by covalent attachment of **ubiquitin**, a small, compact protein that is highly conserved.

**N-end rule**: On average, a protein’s **half-life** correlates with its **N-terminal residue**.
- Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours.
- Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.

**PEST** proteins, rich in Pro (P), Glu (E), Ser (S) and Thr (T), are more rapidly degraded than other proteins.

Protein expression – RNA levels influence protein production
Total levels of protein is a balance of both degradation and production
Availability

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