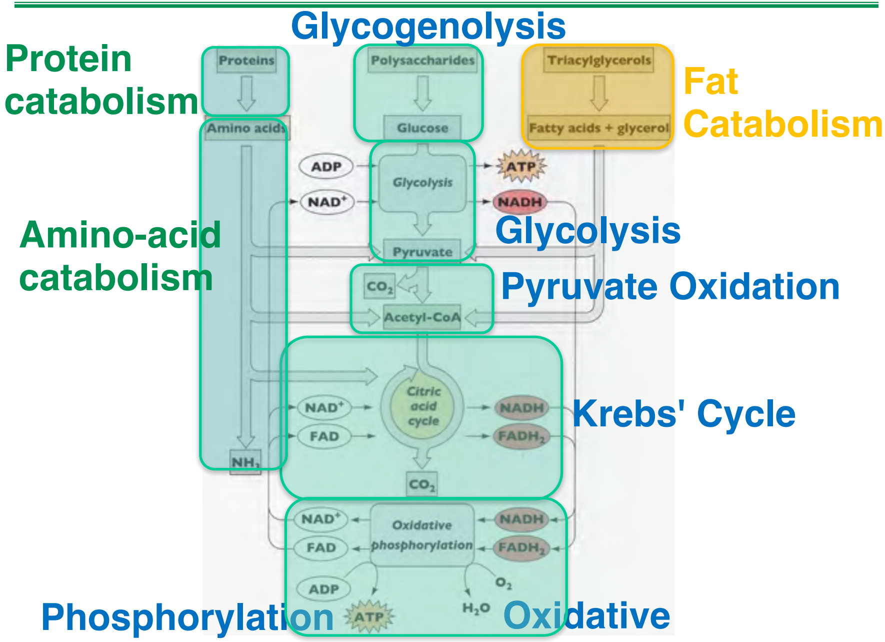
Nitrogen Catabolism



CATABOLISM III:

Digestion and utilization of proteins

- Protein degradation
- Protein turnover
  - The ubiquitin pathway
  - Protein turnover is tightly regulated
- Elimination of nitrogen
  - By fish, flesh and fowl
  - How is the N of amino acids liberated and eliminated?
- How are amino acids oxidized for energy

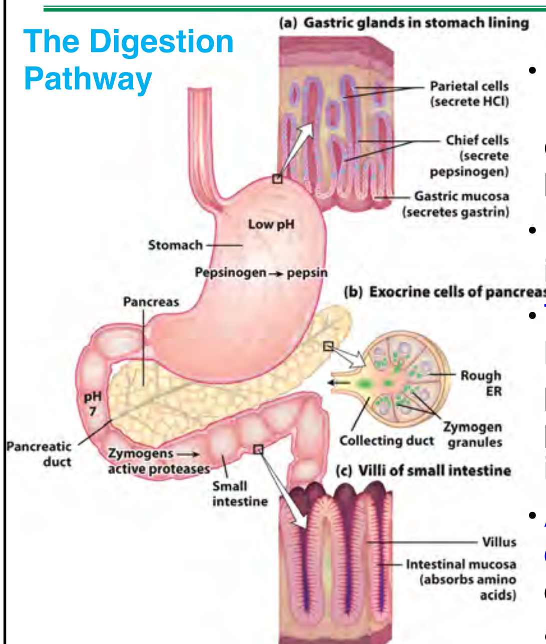
Protein Catabolism

Sources of AMINO ACIDS:

- Dietary amino acids that exceed body's protein synthesis needs
- Excess amino acids from protein turnover (e.g., proteolysis and regeneration of proteins)
- Proteins in the body can be broken down (muscle wasting) to supply amino acids for energy when carbohydrates are scarce (starvation, diabetes mellitus).
- Carnivores use amino acids for energy more than herbivores, plants, and most microorganisms

Protein Catabolism

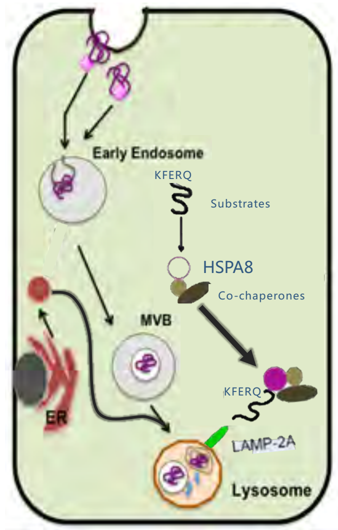
The Digestion Pathway



- Pro-enzymes are secreted (zymogens) and the environment activates them by specific proteolysis.
- Pepsin hydrolyzes protein into peptides in the stomach.
- Trypsin and chymotrypsin hydrolyze proteins and larger peptides into smaller peptides in the small intestine.
- Aminopeptidase and carboxypeptidases A and B degrade peptides into amino acids in the small intestine.

Protein Catabolism

The Lysosomal Pathway



- Endocytosis, either receptor-mediated, phagocytosis, or pinocytosis engulfs extra-cellular proteins into vesicles.
- These internal vesicles fuse as an early endosome.
- This early endosome is acidified by the vATPase ("v" for vesicular).
- Components that are recycled, like receptors, are sequestered in smaller vesicles to create the multivesicular body (MVB), sometimes called a late endosome.
- If set for degradation, it will fuse with a primary lysosome (red) which contains many types of cathepsin proteases.
- In the secondary lysosome, proteins (and other macromolecules) are hydrolyzed into amino acids and internalized into the cell.

Some **cellular** proteins are degraded if they have a KFERQ motif through a chaperone-mediated autophagy pathway…... This leads us to the main mechanism of cellular-protein turnover..........

Protein Catabolism

Protein Turnover (within cell):

- Half-lives of proteins range from seconds to days to even months.

Examples:

- Hemoglobin is long lived.
- Defective proteins are short lived, as are many regulatory proteins that respond to rapidly changing needs (e.g., cyclins).

- But all are eventually degraded; **Protein Degradation Is Inevitable**

TABLE 27-9 Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue

Amino-terminal residue	Half-life[a]
Stabilizing	
Ala, Gly, Met, Ser, Thr, Val	> 20 h
Destabilizing	
Gln, Ile	~30 min
Glu, Tyr	~10 min
Pro	~7 min
Asp, Leu, Lys, Phe	~3 min
Arg	~2 min

Source: Information from A. Bachmair et al., *Science* 234:179, 1986.
[a]Half-lives were measured in yeast for the β-galactosidase protein modified so that in each experiment it had a different amino-terminal residue. Half-lives may vary for different proteins and in different organisms, but this general pattern appears to hold for all organisms.

Alexander Varshavsky

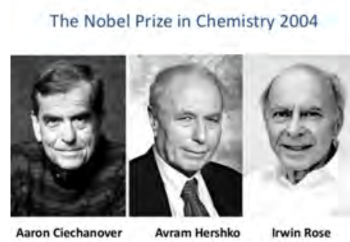
What is this ubiquitination?

N-end rule
Found it was ATP dependent
Found a protein associated:
called it ubiquitin
The N-end rule applied to ubiquitination

Protein Catabolism

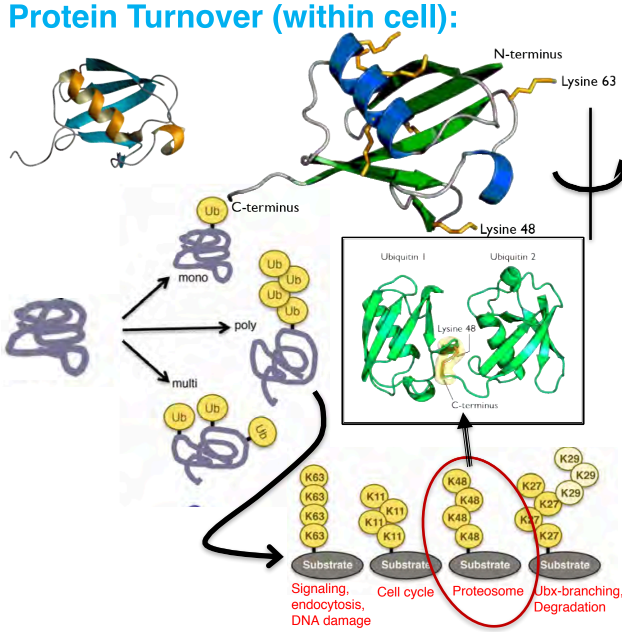
Protein Turnover (within cell): Ubiquitination

- In eukaryotes, proteins are linked to the protein **ubiquitin**; found everywhere in all euk cells (hence the name)
  - Ubiquitin is very highly conserved among all eukaryotes.
  - Only 3 substitutions among all species
  - Works via activating enzyme E1, conjugating enzyme E2, and ligating enzyme E3
- Ubiquinated proteins are cleaved by the 26 S proteasome complex.
  - The proteosome is ATP-dependent.
- In *E. coli*, **Lon** (for "long form," an ATP-dependent protease) hydrolyzes defective or short-term peptides.
  - CLp-P (20S), CLp-A (19S), CLpX



Protein Catabolism

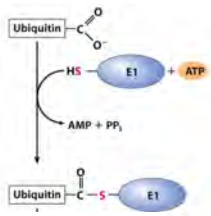
Protein Turnover (within cell):



- Ubiquitin is a 8600 Da protein of 76 residues
- Ubiquitin can add to lysines on targeted (N-end rule) proteins using its C-term carboxylate to make an amide (isopeptide) linkage
- There are seven different Lys residues used
- Proteins are either mono, poly, or multi-ubiquinated.
- The most important one is K48 poly ubiquination.

Protein Catabolism

Protein Turnover (within cell):

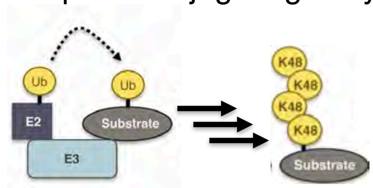


Attachment of Ubiquitin to Target Proteins

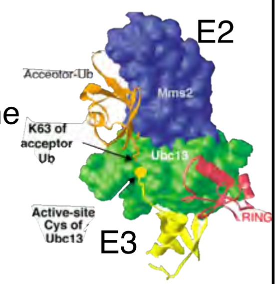
What does this
reaction look like?

E1=Ubiquitin-activating enzyme

E2=Ubiquitin-conjugating enzyme



E3=Ubiquitin-ligase
- Substrate selection
- >600 different E3 ligases in humans

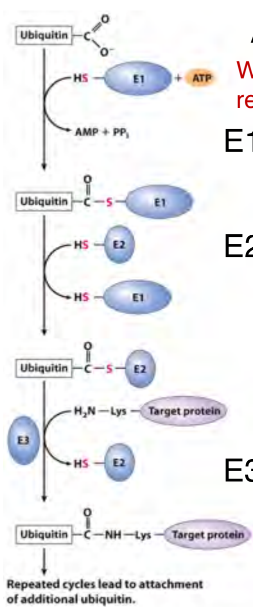


MMS2-**UBC13** ubiquitin ligase
complex from yeast

Dr. Kornberg: **Lecture 03.03.17**
(26:04-28:05)-Pirates
(2 min)

Protein Catabolism

Protein Turnover (within cell):



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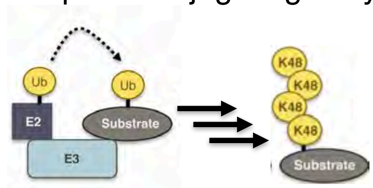

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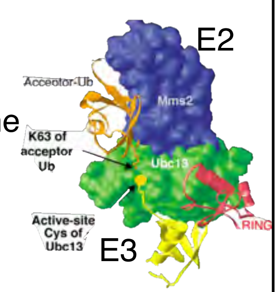

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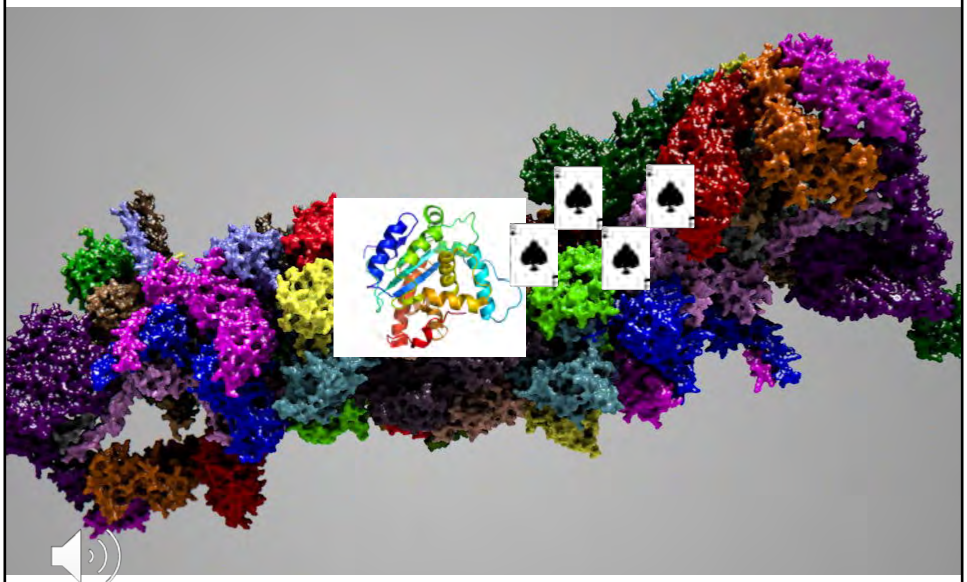
Protein Catabolism

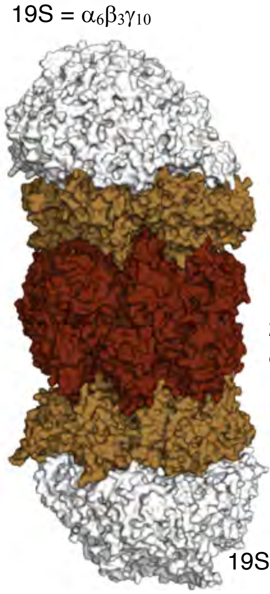
Protein Turnover (within cell):



Protein Catabolism

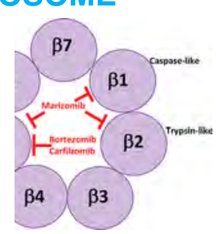
Protein Turnover (within cell): THE 26S PROTEOSOME

19S = $\alpha_6\beta_3\gamma_{10}$



20S = $\alpha_7\beta_{14}\alpha_7$

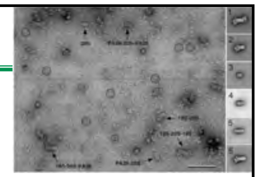
19S = $\alpha_6\beta_3\gamma_{10}$

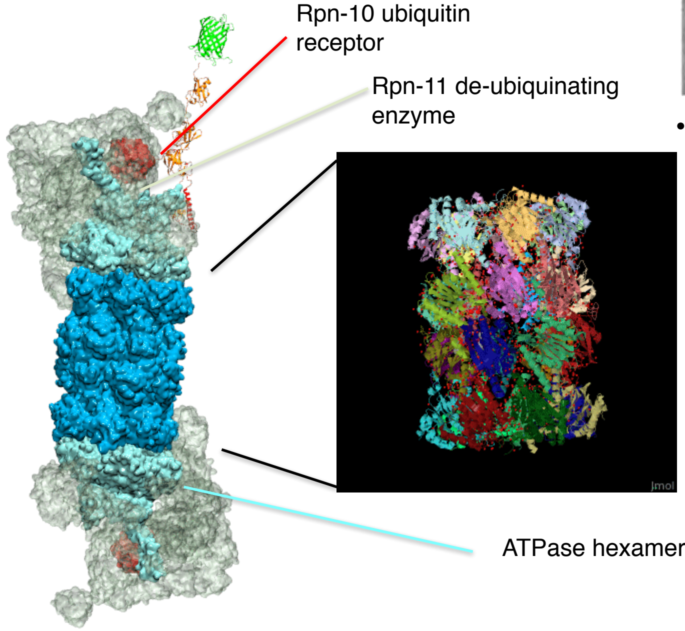


20S does the protease. The β subunits are isozymes; proteases with different specificities

ıes ATP hydrolysis
ₓ recognition (γ),
folding (γ).

Protein Catabolism





- Cryo-EM was used to attain these structures within the last 2 years.

Protein Catabolism

Fate of Amino Acids:

Protein Biosynthesis | Oxidation

Once broken down to amino acid, all types of protein are treated the same way depending on the organism's energy needs:

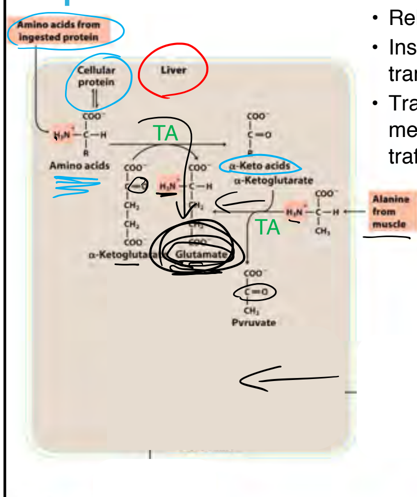
1. Recycled into new proteins
2. Oxidized for energy
   - There are two separate aspects:
     - removal of amino group (urea cycle)
     - entry into central metabolism (glycolysis, citric acid cycle)

We'll call this "Amino Acid Degradation or Catabolism"

Amino Acid Catabolism

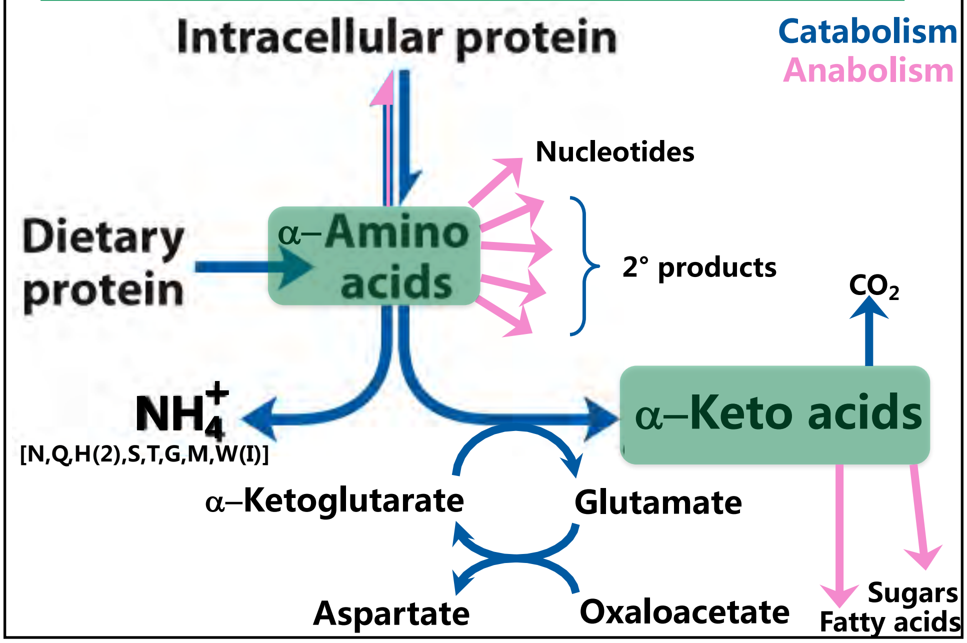
Amino Acid Catabolism

Step 1: Removal of the Amino Group



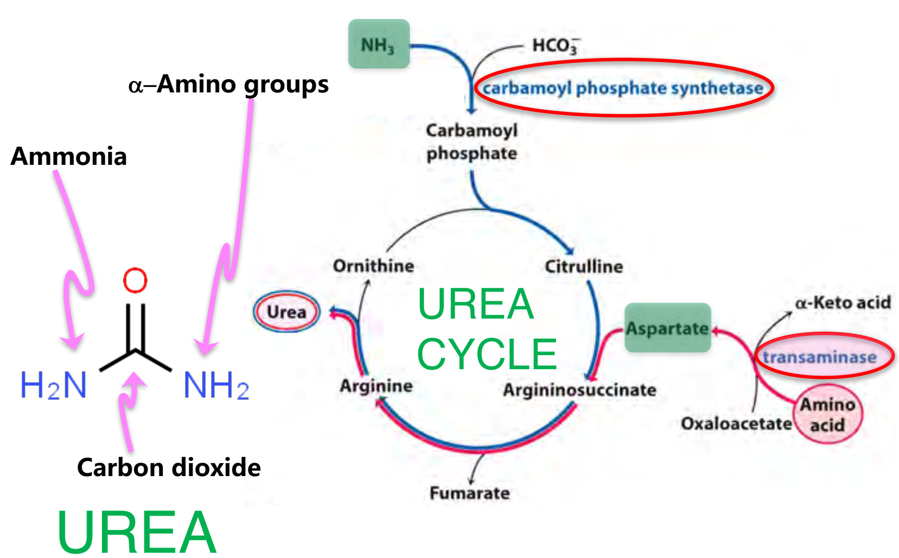
- Release of free ammonia, which is toxic.
- Instead of Ammonia, nitrogen is captured by a series of trans-aminations (TA).
- Transaminations allow transfer of an amine to a common metabolite (e.g., α-ketoglutarate) and generate a traffickable amino acid (e.g., glutamate).
  - Catalysis by aminotransferases (TA):
  - Uses the pyridoxal phosphate (PLP) cofactor
  - Typically, α-ketoglutarate accepts amino groups.
    - Transfer of one amine to α-ketoglutarate results in synthesis of glutamate (e.g., transamination).
    - Transfer of a second amine results in synthesis of glutamine (e.g., glutamine synthetase).
  - L-Glutamine acts as a temporary storage of nitrogen and a way of de-toxifying the ammonia
  - L-Glutamine can donate the amino group when needed for amino acid biosynthesis.

Amino Acid Catabolism



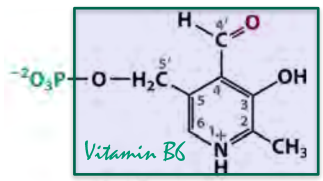
Amino Acid Catabolism

What is the fate of the free ammonia and Asp?

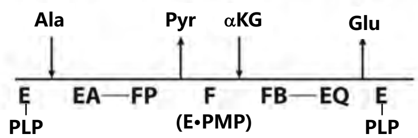


Amino Acid Catabolism: Transamination

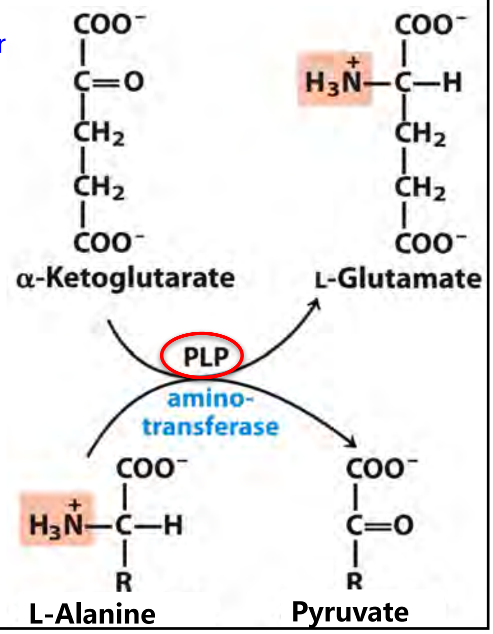
- Catalyzed by aminotransferases
- Uses the pyridoxal phosphate cofactor
- Typically, either oxaloacetate or α-ketoglutarate accept amino groups.
  - Transfer of the α-amino group to α-ketoglutarate results in synthesis of glutamate (e.g., transamination).



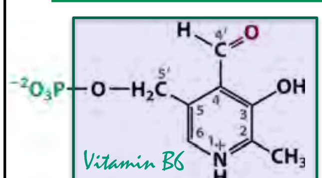
Pyridoxal-5′-phosphate (PLP)



Ping-pong bi bi



Amino Acid Catabolism: Transamination



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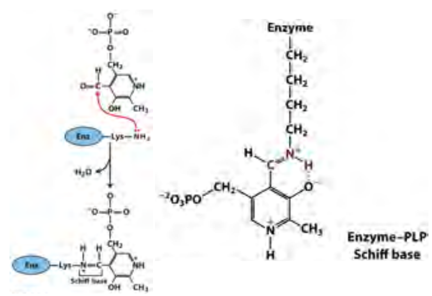
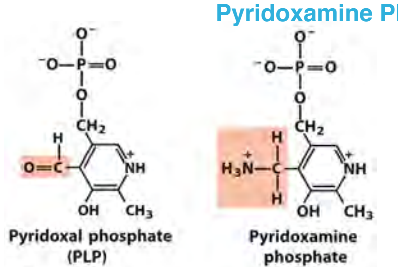


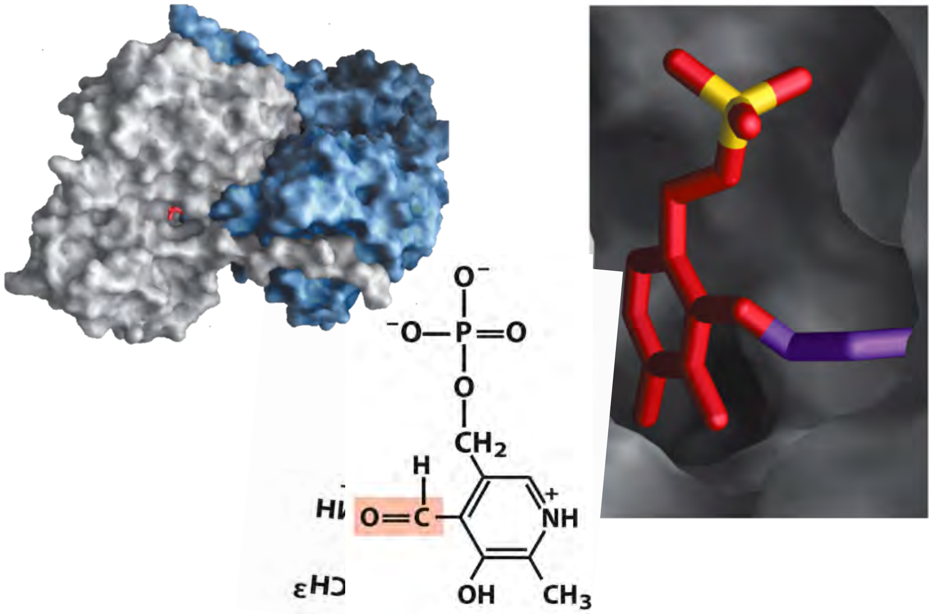
Structure of Pyridoxal Phosphate and Pyridoxamine Phosphate



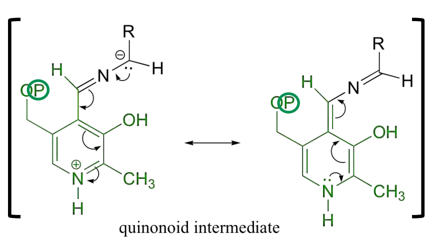
- Intermediate, enzyme-bound carrier of amino groups
- The linkage is made via a nucleophilic attack of the amino group of an active-site lysine.
- Making an internal aldimine

- Aldehyde form can react reversibly with amino groups.
- Aminated form can react reversibly with carbonyl groups to make a Schiff base.

Amino Acid Catabolism: Transamination

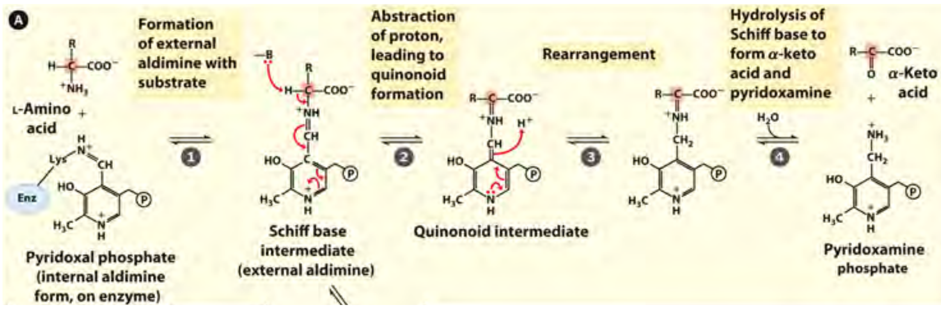


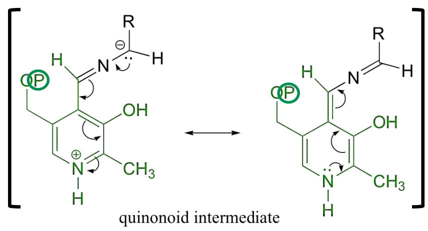
Amino Acid Catabolism: Transamination



Amino Acid Catabolism: Transamination

See Sapling animated Figure(a)





quinonoid intermediate

http://media.saplinglearning.com/priv/he/lehninger/aem/1806a_pyridoxal_phosphate.html