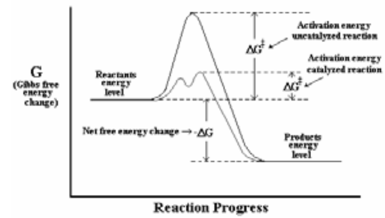
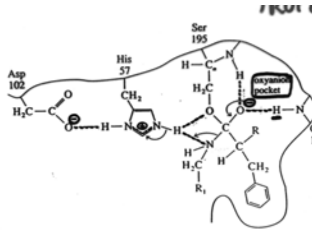
Lecture 6

Protein function and enzyme kinetics

Enzymes lower the energy of activation



Transition State Stabilization by Chymotrypsin

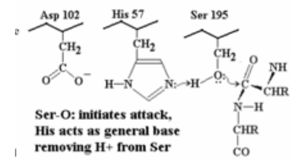


- **Structure of ts stabilized**
- **Stability → lower energy**
- **Lower energy → decreases ΔG***
- **Decreased ΔG* → increased rate**

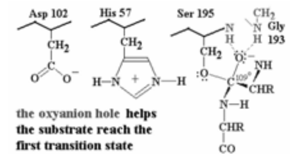
The substrate binds with its target C=O group next to Ser 195. Substrate is shown in blue. The catalytic triad, which is part of chymotrypsin, is in black. Electron exchanges are in red.

Cooperative action among the **catalytic triad** amino acids, helps form the **first transition state**.

His 57 acts as a **general base** by removing a proton from Ser-OH, and this helps the Ser O: make its nucleophilic attack on the substrate C=O, while negative Asp 102 promotes formation of HisH+.



The **oxyanion hole** is also part of chymotrypsin, and consists of the backbone -NH- groups of Gly 193 and Ser 195 (shown in pink). The N-H groups are positioned in such a way that they will donate strong H-bonds to the substrate C=O, if the C atom is **tetrahedral, as found in the transition state**. This strains the bonds of the trigonal planar C=O of the original substrate, helping the reaction to proceed to the transition state.



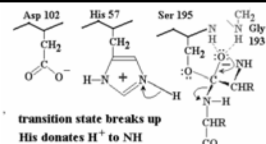
Breakdown of the first transition state and formation of the intermediate:

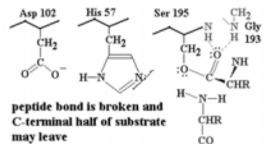
The transition state is broken up by loss of the peptide bond after the target amino acid.

Histidine now acts as a **general acid** donating its proton to the N atom so that it is a better leaving group.

After gaining a proton from His 57, a neutral NH_2- group is formed, so the C-terminal half of the substrate is no longed bonded to the enzyme and is now **free to leave.**

The remainder of the substrate **remains bonded via its carboxylate group in an ester bond to Ser 195**. This is called the **acyl-enzyme intermediate.**

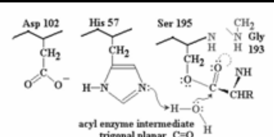




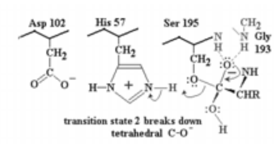
Step 2

With half of the substrate out of the way, there's now room for an H_2O to get into range of the catalytic triad.
His 57 acts as a **general base to steal a proton** from H_2O, enhancing the nucleophilic power of the O in H_2O (much like the earlier step with Ser 195).

H_2O can now attack the substrate

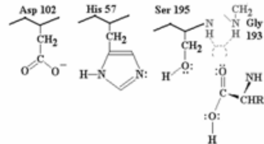


Transition state 2 breaks up by HisH+ 57 acting as a **general acid to donate the proton back to Ser 195**, in turn breaking the Ser-O to substrate C--O ester bond.



Final result:

The N-terminal half of the original peptide substrate now carries the target amino acid at its newly formed C-terminus. This is now free to leave in the form of a carboxylic acid, since the C=O is trigonal planar and pops out of the oxyanion hole.



Proteins as enzymes

There are 6 major classes of enzymes:

1. Oxidoreductases, which are involved in oxidation, reduction, and electron or proton transfer reactions;
2. Transferases, catalyzing reactions in which groups are transferred;
3. Hydrolases that cleave various covalent bonds by hydrolysis;
4. Lyases catalyze reactions forming or breaking double bonds;
5. Isomerases catalyze isomerization reactions;
6. Ligases join constituents together covalently.

Enzymes fall into classes based on function

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Enzyme Kinetics

- Enzymes are protein catalysts that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process.

Enzyme reaction rates are determined by several factors.

- the concentration of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated [S] and is expressed in unit of molarity.
- the temperature. As the temperature rises, molecular motion - and hence collisions between enzyme and substrate - speed up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes denatured and ineffective.

Enzymes cont.

- the presence of inhibitors.
  - **competitive inhibitors** are molecules that bind to the same site as the substrate - preventing the substrate from binding as they do so - but are not changed by the enzyme.
  - **noncompetitive inhibitors** are molecules that bind to some other site on the enzyme reducing its catalytic power.
- pH. The conformation of a protein is influenced by pH and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected.

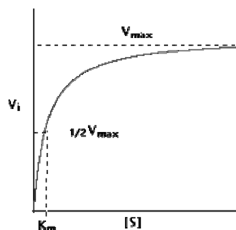
How we determine how fast an enzyme works

- We set up a series of tubes containing graded concentrations of substrate, [S] . At time zero, we add a fixed amount of the enzyme preparation.
- Over the next few minutes, we measure the concentration of product formed. If the product absorbs light, we can easily do this in a spectrophotometer.
- Early in the run, when the amount of substrate is in substantial excess to the amount of enzyme, the rate we observe is the initial velocity of Vi.

Mechaelis Menton kinetics

- Plotting Vi as a function of [S], we find that
- At low values of [S], the initial velocity,Vi, rises almost linearly with increasing [S].
- But as [S] increases, the gains in Vi level off (forming a rectangular hyperbola).
- The asymptote represents the maximum velocity of the reaction, designated Vmax
- The substrate concentration that produces a Vi that is one-half of Vmax is designated the Michaelis-Menten constant, Km(named after the scientists who developed the study of enzyme kinetics).
- Km is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the Km, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

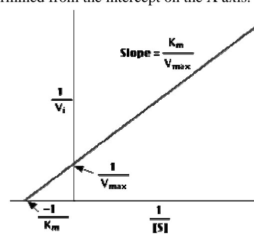
Plotting out our data it might look like this.



V_i = initial velocity (moles/time)
[S] = substrate concentration (molar)
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one-half V_{max} (Michaelis-Menton constant)

Lineweaver-Burke plot

Plotting the reciprocals of the same data points yields a "double-reciprocal" or Lineweaver-Burk plot. This provides a more precise way to determine Vmax and Km. Vmax is determined by the point where the line crosses the 1/Vi = 0 axis (so the [S] is infinite). Note that the magnitude represented by the data points in this plot decrease from lower left to upper right. Km equals Vmax times the slope of line. This is easily determined from the intercept on the X axis.



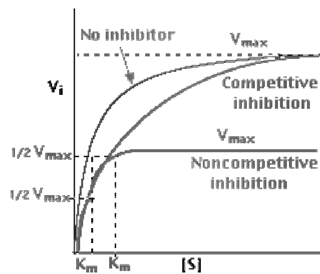
Competitive inhibitors

- Enzymes can be inhibited competitively, when the substrate and inhibitor compete for binding to the same active site or noncompetitively, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency.
- The distinction can be determined by plotting enzyme activity with and without the inhibitor present.
- Competitive Inhibition
- In the presence of a competitive inhibitor, it takes a higher substrate concentration to achieve the same velocities that
- were reached in its absence. So while Vmax can still be reached if sufficient substrate is available, one-half Vmax requires a higher [S] than before and thus Km is larger.

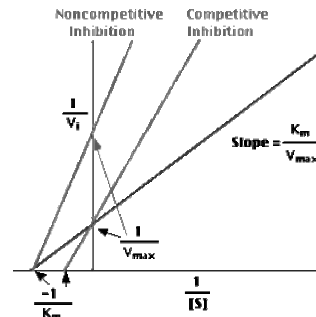
Non-competitive inhibitor

- With noncompetitive inhibition, enzyme molecules that have been bound by the inhibitor are taken out of the game so enzyme rate (velocity) is reduced for all values of [S], including Vmax and one-half Vmax but
- Km remains unchanged because the active site of those enzyme molecules that have not been inhibited is unchanged.

Competitive/noncompetitive inhibitor



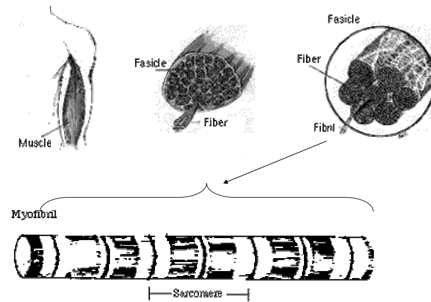
Effect of inhibitors



How do proteins function?

- Structural: Actin is an example it is a major component of the cells architecture as well as the contractile apparatus
- Carriers: Hemoglobin is an example. It functions to carry O_2 to tissue and eliminate CO_2
- Regulatory: Transcription factors bind to DNA a control the level of mRNA that is produced
- Transport: EGFR-epithelial growth factor receptor. Binds EGF and signals for cell growth.
- Binders: Immunoglobulin proteins or antibodies-bind to foreign proteins and destroy infectious agents.

Actin and myosin: the contractile apparatus



Skeletal Muscle Cells

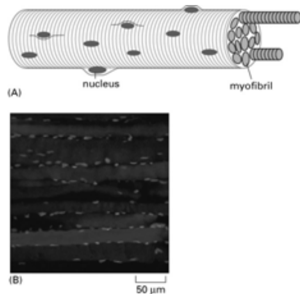
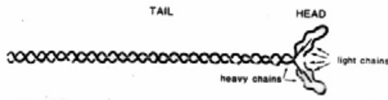


Figure 16-68. Molecular Biology of the Cell, 4th Edition.

Skeletal Muscle Structure

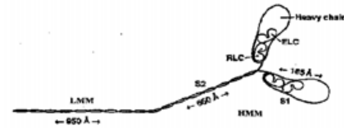
- Muscle cells are formed by fusion of myoblasts
- Myofibrils are parallel arrays of long cylinders packed in the muscle cell
- Sarcomeres are symmetric repeating units from z-line to z-line in the myofibril
- Thick filaments are myosin filaments
- Thin filaments are actin filaments

Structure of Myosin



Myosin is a large asymmetric molecule, it has a long tail and two globular heads (Fig. M1). The tail is about 1,600 Å long and 20 Å wide. Each head is about 165 Å long, 65 Å wide and 40 Å deep at its thickest part. The molecular weight of myosin is about 500,000. In strong denaturing solutions, such as 5 M guanidine-HCl or 8 M urea, myosin dissociates into six polypeptide chains: two heavy chains (molecular weight of each heavy chain about 200,000) and four light chains (two with a molecular weight of 20,000, one with 15,000 and another with 25,000). The two heavy chains are wound around each other to form a double helical structure. At one end both chains are folded into separate globular structures to form the two heads. In the muscle, the long tail portion forms the backbone of the thick filament and the heads protrude as crossbridges toward the thin filament. Each head contains two light chains.

More myosin structure



More details of the myosin structure. When myosin is exposed to the proteolytic enzyme trypsin, fragmentation occurs in the middle of the tail yielding heavy meromyosin (HMM, molecular weight about 350,000) and light meromyosin (LMM, molecular weight about 150,000) HMM containing the head and a short tail can be further split by proteolytic enzymes, such as papain, into subfragment 1 (S1, molecular weight about 110,000) and subfragment 2 (S2). The regions of proteolytic fragmentation may serve as hinges. HMM and S1 bind actin, hydrolyze ATP and are water-soluble. LMM has no sites for actin or ATP binding, but inherits the solubility of myosin in 0.6 M KCl and the self-assembling property of myosin in 0.03 M KCl. S2 is water-soluble. Myosin and its proteolytic fragments can be visualized by electron microscopy

Arrangement of Myosin Molecules in Thick Filaments

- bipolar polymer of myosin
- myosin tails align and point to center of sarcomere
- myosin heads arranged in a helical pattern pointing away from center
- myosin heads reach out from the thick filaments to contact the actin filaments
- contain ~300 molecules of myosin

Myosin filament

Thin Filaments

- actin filaments in the sarcomere are of fixed length
- actin filaments are cross-linked by α-actinin at Z-line
- both ends of actin filaments are capped
- barbed ends are embedded at the Z-line
- tropomyosin and troponins bind along each filament

Structure of actin filament

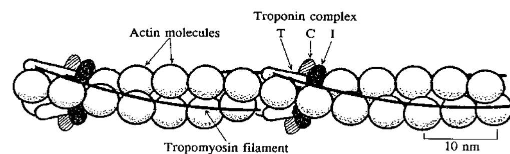
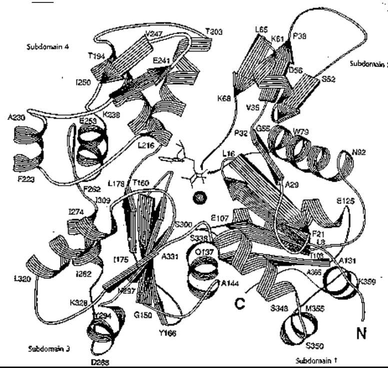


Figure 11.9 Schematic drawing of part of an actin filament showing the relationship between successive pairs of actin molecules, thin filaments of tropomyosin, and the three types of troponin. Another depiction of a (nonmuscle) actin filament is given in Figure 2.4A.

Actin in detail



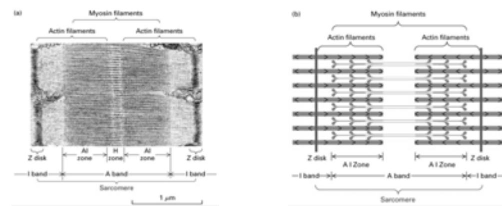
Actin structure

- Folding of the actin molecule is represented by ribbon tracing of the a-carbon atoms. N and C correspond to the amino- and carboxyl-terminals, respectively. The letters followed by numbers represent amino acids in the polypeptide chain. A hypothetical vertical line divides the actin molecule into two domains "large", left side, and "small", right side. ATP and Ca2+ are located between the two domains. These two domains can be subdivided further into two subdomains each, the small domain being composed of subdomains 1 and 2, and the 2 has significantly less mass than the other three subdomains and this is the reason of dividing actin into small and large domains). The four subdomains are held together and stabilized mainly by salt bridges and hydrogen bonds to the phosphate groups of the bound ATP and to its associated Ca2+ localized in the center of the molecule.

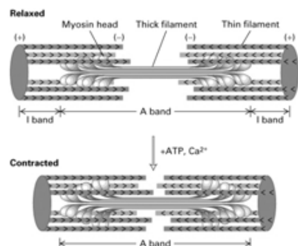
Actin domains

- 1. Where does it polymerize with actin?
- 2. Where does it interact with troponin and tropomyosin?
- 3. Where does it interact with myosin?
- 4. How could we answer this question?

Structure of a Sarcomere

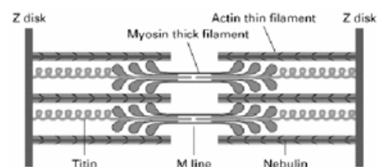


Muscle Contraction

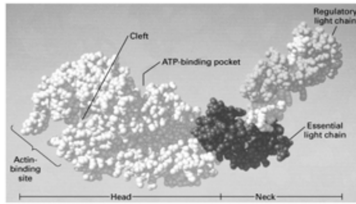


Neither thick or thin filaments change length during muscle contraction, only the overlap between them changes, leading to changes of sarcomere length (z- to z distance)

Stabilization of the Alignment of Thick and Thin Filaments



Crystal Structure of Myosin Head and Lever Arm



Regulation of Non-muscle Myosin II Assembly

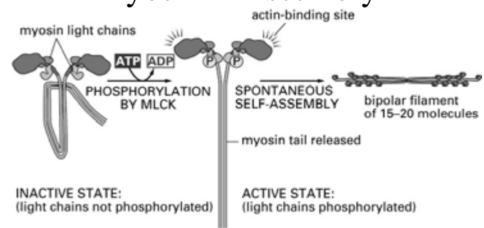
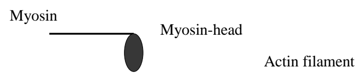


Figure 16–67 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Muscle continue



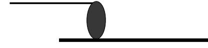
Muscle continue

Muscle continue

Muscle continue

Muscle continue

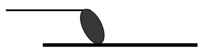
Muscle continue



Muscle continue

Muscle continue

Muscle continue



Myosin Superfamily

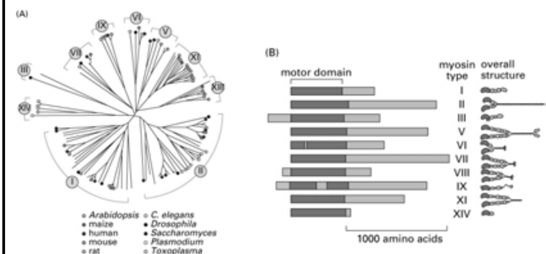
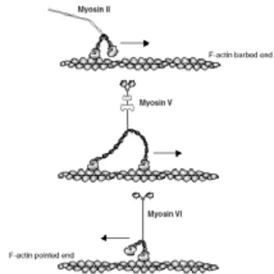


Figure 16-54 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 16-54 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Three examples of the diverse structures of members of the myosin superfamily



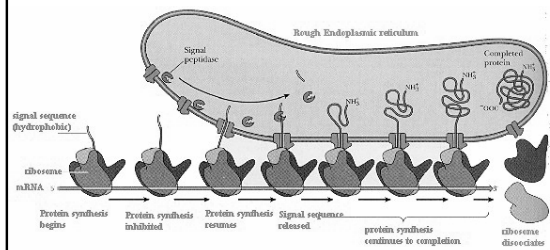
Three examples of the diverse structures of members of the myosin superfamily.

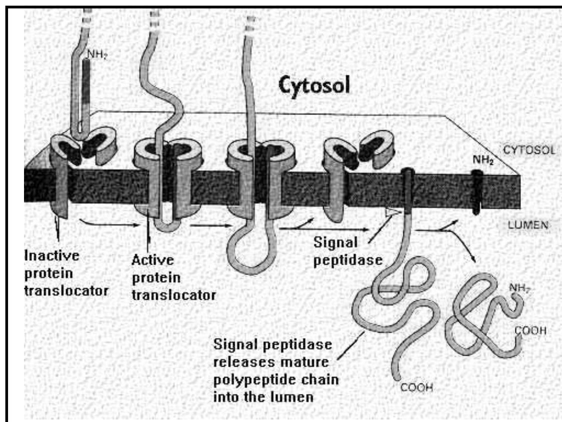
In vitro Motility Assay

1. Attach myosin S1 on the cover slip
2. Add fluorescently tagged actin filament
3. Addition of ATP initiates the movement of the filaments
4. Also done by coating cover slip with actin filaments and use fluorescently tagged myosin motor domain

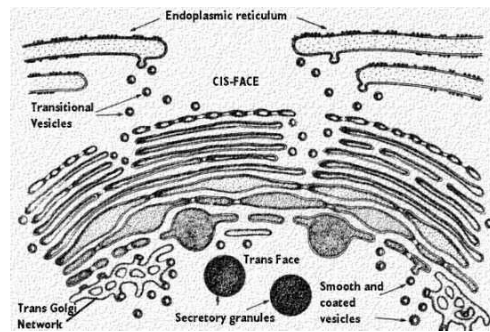
In vitro motility assay

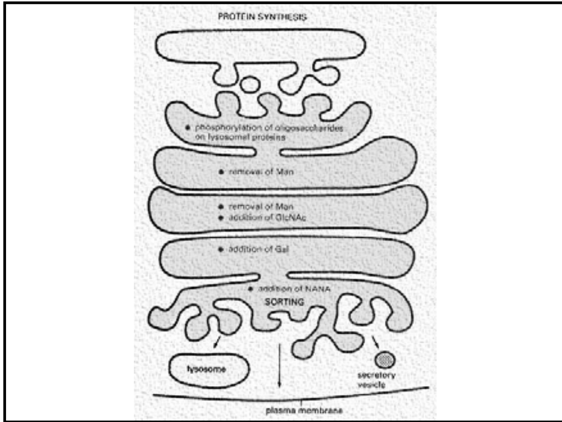
Proteins that are going to be modified are targeted for synthesis on the endoplasmicreticulum by a signal peptide

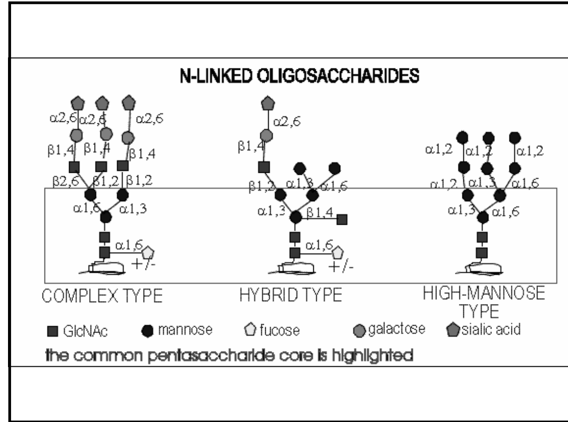


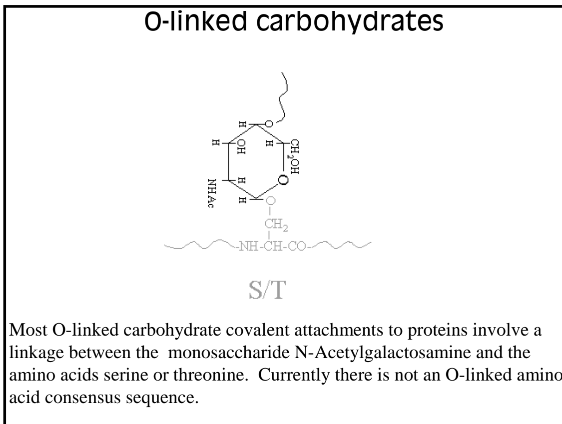


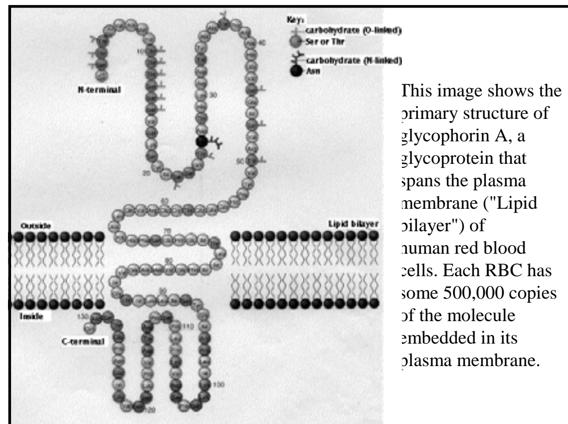
Once in the ER the protein is targeted to the golgi for modification




PROTEIN SYNTHESIS
phosphorylation of oligosaccharides on lysosomal proteins
removal of Man
removal of Man
addition of GlcNAc
addition of Gal
addition of NANA
SORTING
lysosome
secretory vesicle
plasma membrane


N-LINKED OLIGOSACCHARIDES
COMPLEX TYPE
HYBRID TYPE
HIGH-MANNOSE TYPE
GlcNAc
mannose
fucose
galactose
sialic acid
the common pentasaccharide core is highlighted


O-linked carbohydrates
S/T
Most O-linked carbohydrate covalent attachments to proteins involve a linkage between the monosaccharide N-Acetylgalactosamine and the amino acids serine or threonine. Currently there is not an O-linked amino acid consensus sequence.


N-terminal
Outside
Lipid bilayer
Inside
C-terminal
This image shows the
rimary structure of
lycophorin A, a
lycoprotein that
pans the plasma
membrane ("Lipid
ilayer") of
uman red blood
ells. Each RBC has
ome 500,000 copies
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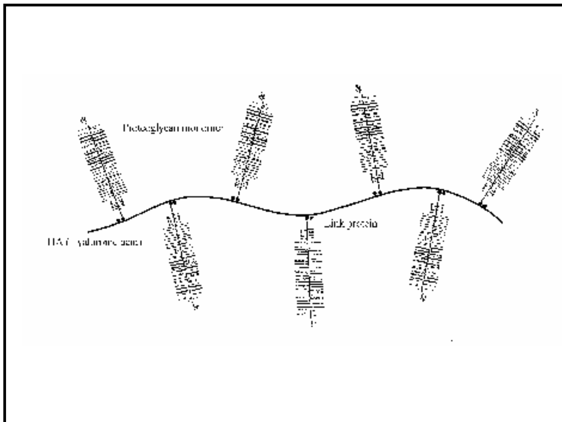
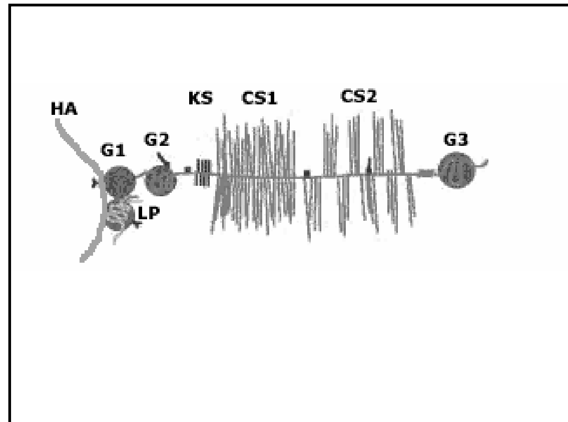
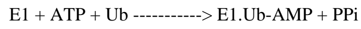
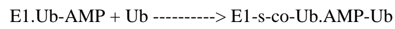
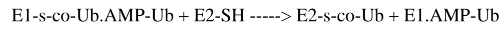


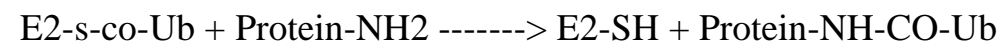

HA
KS
CS1
CS2
G1
G2
G3
LP

Ubiquitin pathway for protein degradation

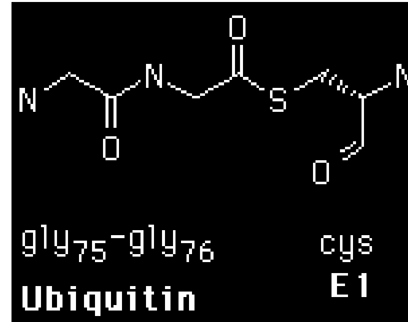








Thioester bond



Isopeptide bond