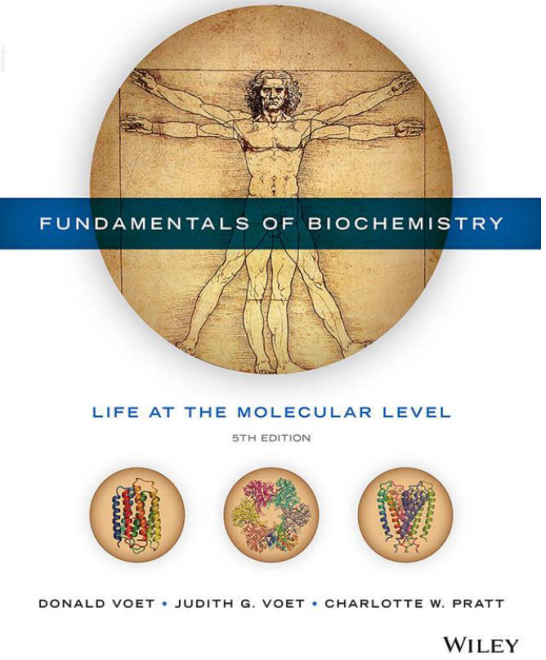
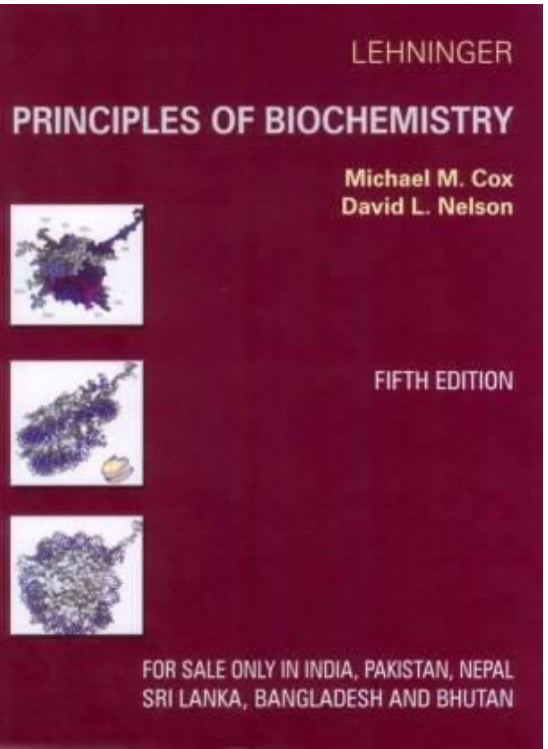


Control of Enzyme Activity



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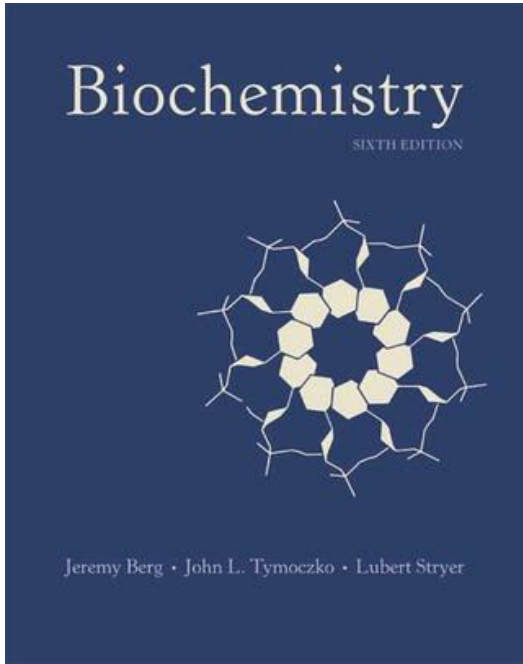
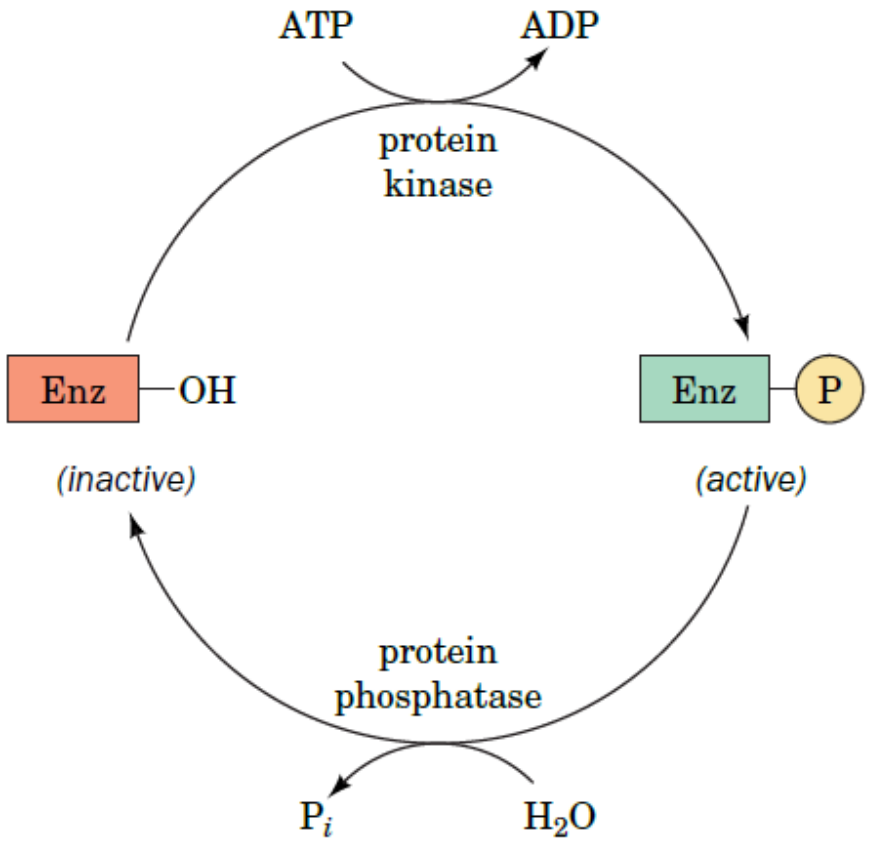
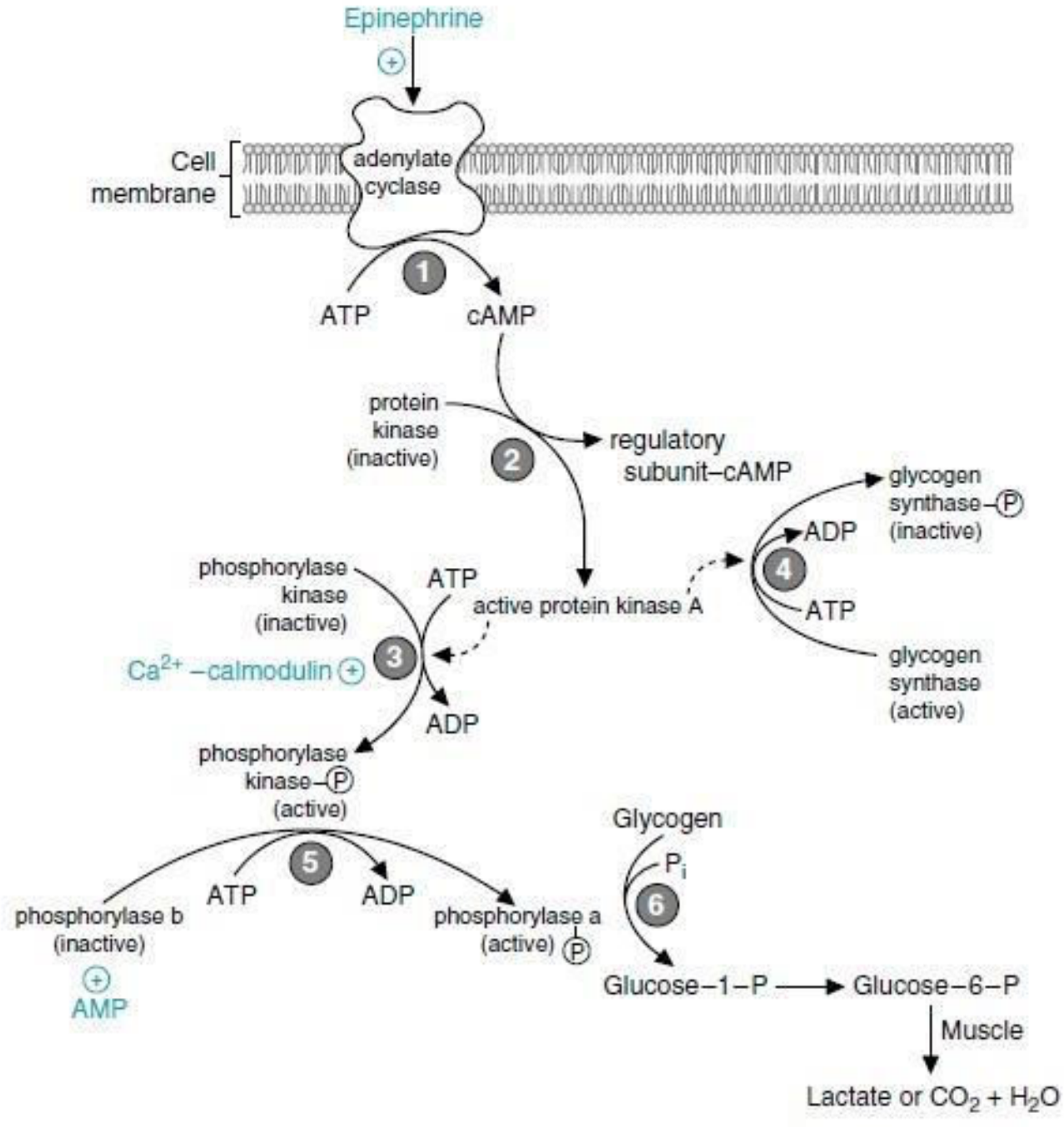


Protein Phosphorylation/dephosphorylation

In addition to allosteric interactions, many enzymes may be subject to control by covalent modification. In eukaryotes, by far the most common such modification is **phosphorylation** and **dephosphorylation** of the hydroxyl group of a Ser, Thr, or Tyr residues.



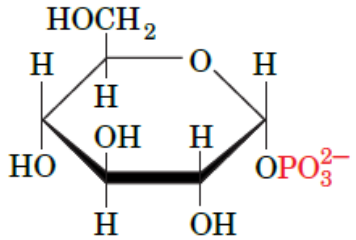

Epinephrine
Cell membrane
adenylate cyclase
1
ATP
cAMP
protein kinase (inactive)
2
regulatory subunit–cAMP
glycogen synthase–P (inactive)
ADP
4
ATP
glycogen synthase (active)
phosphorylase kinase (inactive)
ATP
active protein kinase A
Ca2+ –calmodulin
3
ADP
phosphorylase kinase–P (active)
Glycogen
Pi
5
ATP
ADP
6
phosphorylase b (inactive)
AMP
phosphorylase a (active) P
Glucose–1–P
Glucose–6–P
Muscle
Lactate or CO2 + H2O

- Such enzymatic modification processes, which are catalyzed by enzymes known as **protein kinases** and **protein phosphatases**, alter the activities of the modified proteins.

- Indeed, ~30% of human proteins, which collectively participate in nearly all biological processes, are subject to control by reversible phosphorylation.

- As an example of an enzyme whose activity is controlled by covalent modification, let us consider **glycogen phosphorylase** (or simply phosphorylase), which catalyzes the phosphorolysis of glycogen to yield glucose-1-phosphate (G1P):

$$\underset{(n \text{ residues})}{\text{Glycogen}} + P_i \rightleftharpoons \underset{(n-1 \text{ residues})}{\text{glycogen}} + \text{G1P}$$



Glucose-1-phosphate (G1P)

- This is the **rate-controlling step** in the metabolic pathway of glycogen breakdown, an important supplier of fuel for metabolic activities.

- Mammals express **three isozymes** (catalytically and structurally similar but genetically distinct enzymes from the same organism) of glycogen phosphorylase, those from **muscle, brain, and liver**.

- Muscle glycogen phosphorylase, which we discuss here, is a **dimer of identical 842-residue** subunits.

- It is regulated both by allosteric interactions and by phosphorylation/dephosphorylation.

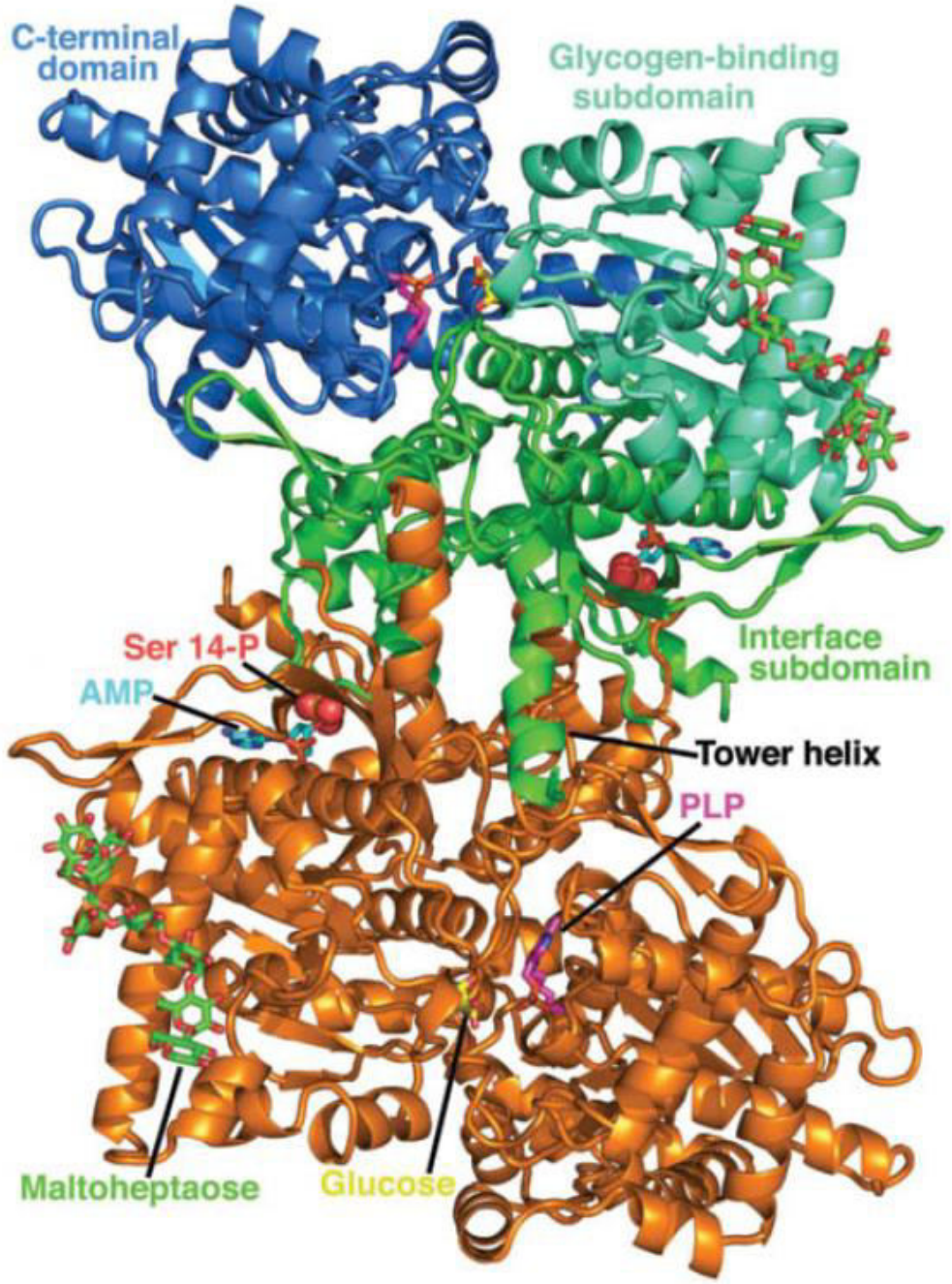
- The phosphorylated form of the enzyme, **phosphorylase a**, has a phosphoryl group esterified to its **Ser14**. The **dephospho form is called phosphorylase b**.

- The X-ray structures of phosphorylase a and phosphorylase b, which were respectively determined by **Robert Fletterick and Louise Johnson**, are similar.

- Both have a large N-terminal domain (484 residues; the largest known domain) and a smaller C-terminal domain.

- The N-terminal domain, which is subdivided into a **glycogen-binding subdomain** and an **interface subdomain**, contains the **phosphorylation site** (Ser 14), an **allosteric effector site**, a **glycogen-binding site** (called the glycogen storage site), and all the inter subunit contacts in the dimer.

- The enzyme's **active site is located at the center** of the subunit.



Phosphorylation and dephosphorylation can alter enzymatic activity in a manner that resembles Allosteric control

- Glycogen phosphorylase has two conformational states, the enzymatically active R state and the enzymatically inactive T state.

- The **T-state enzyme is inactive** because it has a **malformed active site** and a **surface loop** (residues 282–284) that blocks substrate access to its binding site.

- In contrast, in the **R-state enzyme**, the side chain of **Arg 569** has **reoriented** so as to bind the substrate phosphate ion and the **282-284 loop no longer blocks the active site**, thereby permitting the enzyme to bind substrate and efficiently catalyze the phosphorolysis of glycogen.

- The **phosphorylation of Ser14 promotes phosphorylase's T (inactive) → R (active) conformational change**.

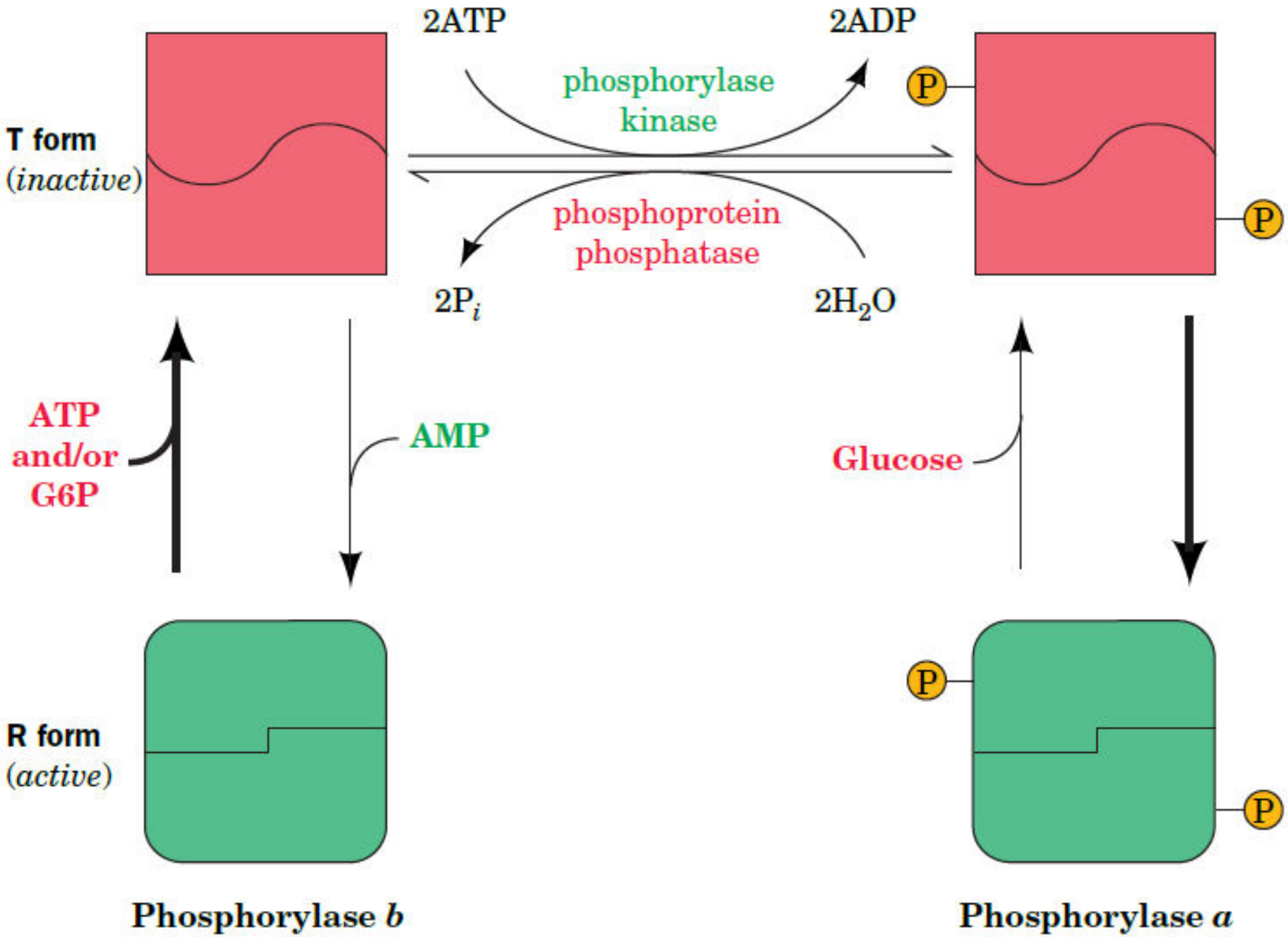
- Moreover, these different enzymatic forms respond to different allosteric effectors.

- Thus **ATP** and **glucose- 6-phosphate** preferentially bind to the T state of **phosphorylase b** and, in doing so, inactivate the enzyme, whereas **AMP** preferentially binds to the R state of phosphorylase b and hence activates it.

- In contrast, **phosphorylase a** only allosteric effector is **glucose, which binds to the enzyme's T state and inactivates the enzyme**.

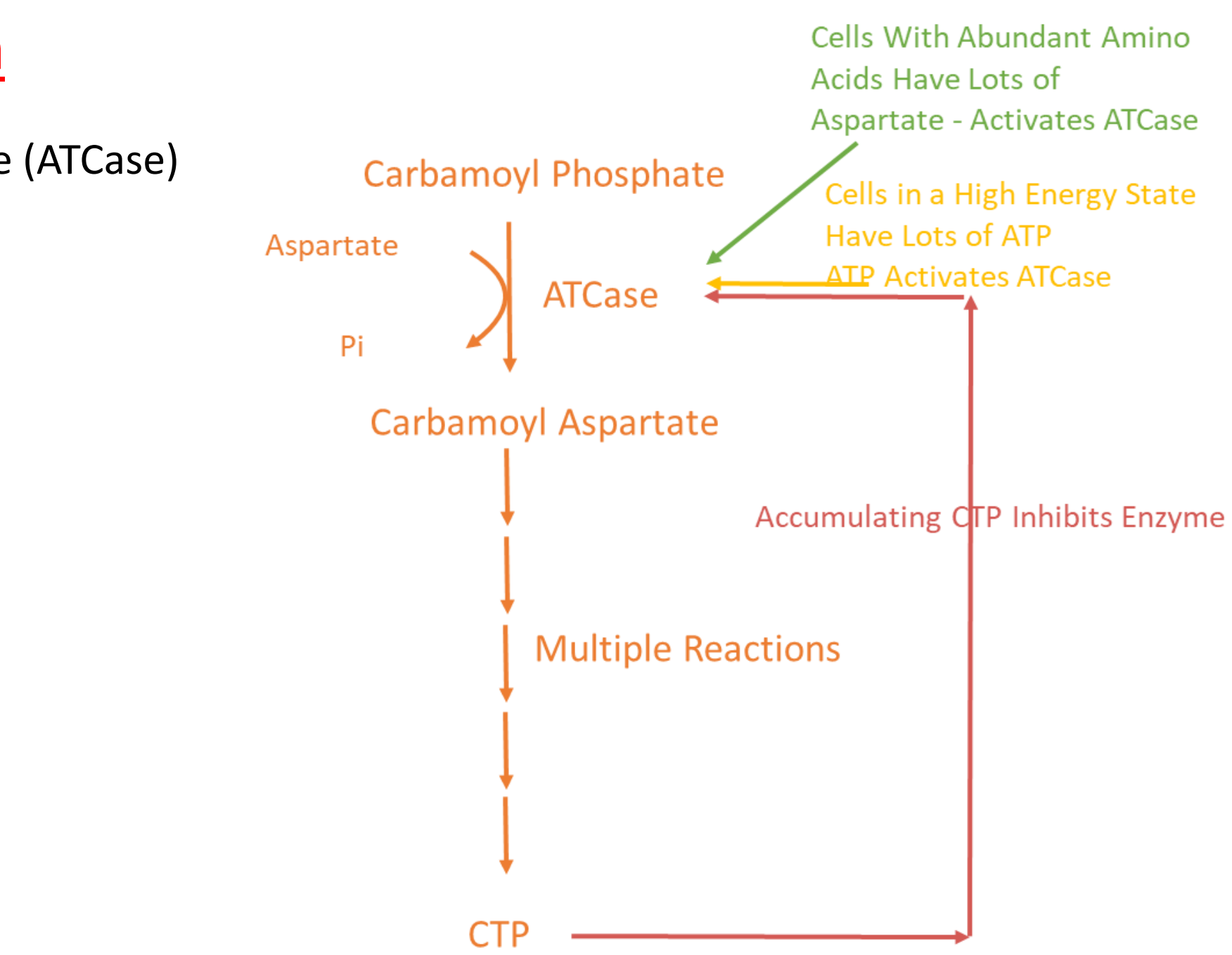
- Note that **ATP, G6P, and glucose are present in relatively high concentrations in muscle under conditions of low exertion**, a state when glycogen breakdown would be superfluous.

- Whereas **AMP is present in relatively high concentration in muscles under conditions of high exertion**, a state when the G1P product of the phosphorylase reaction helps fuel muscle contraction.

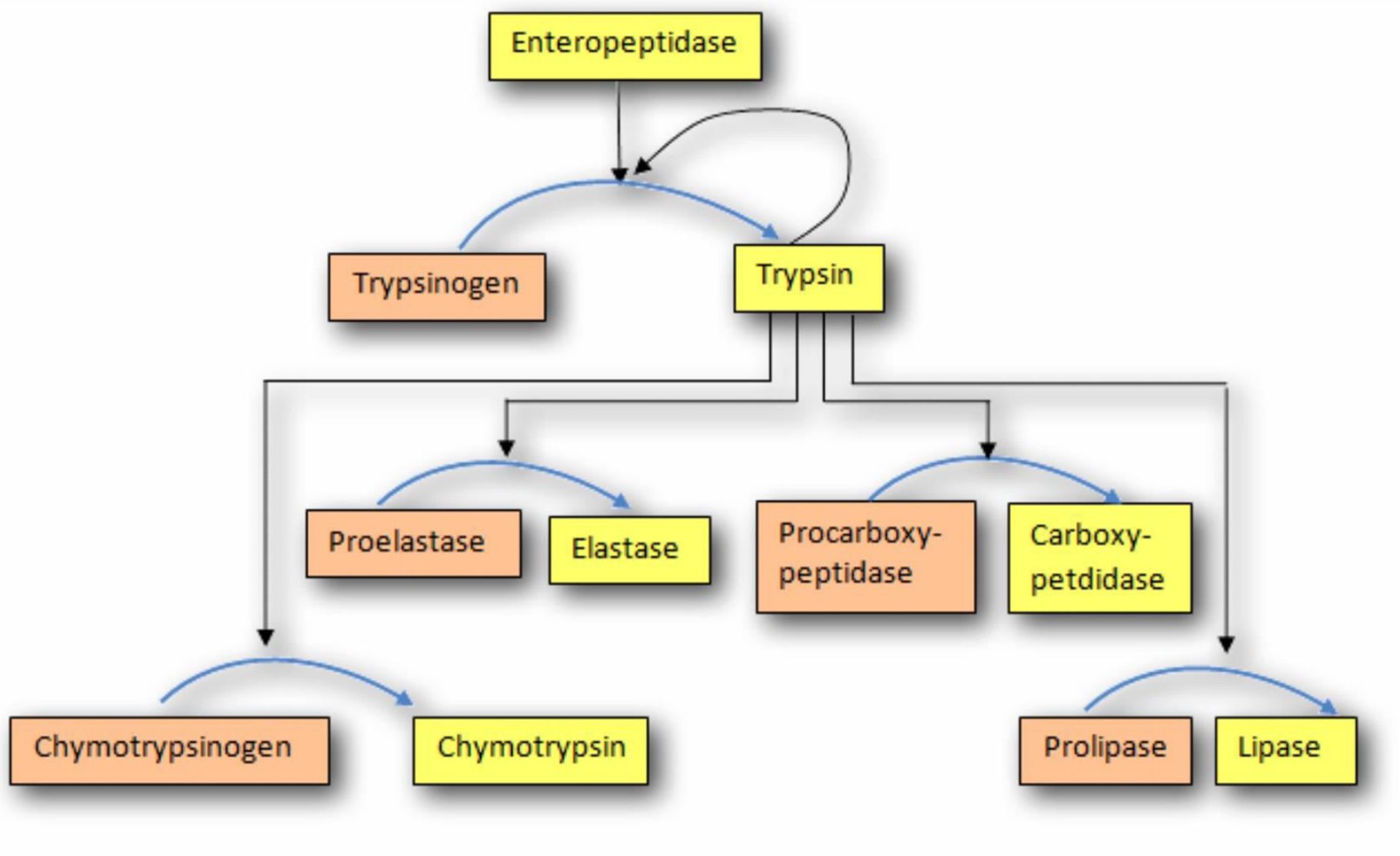

2ATP
2ADP
phosphorylase kinase
T form (inactive)
P
P
phosphoprotein phosphatase
2P$_i$
2H$_2$O
ATP and/or G6P
AMP
Glucose
R form (active)
P
P
Phosphorylase b
Phosphorylase a

Feedback Inhibition

- Aspartate Transcarbamoylase (ATCase)



Zymogen Activation or proteolytic activation



Zymogens Are Inactive Enzyme Precursors

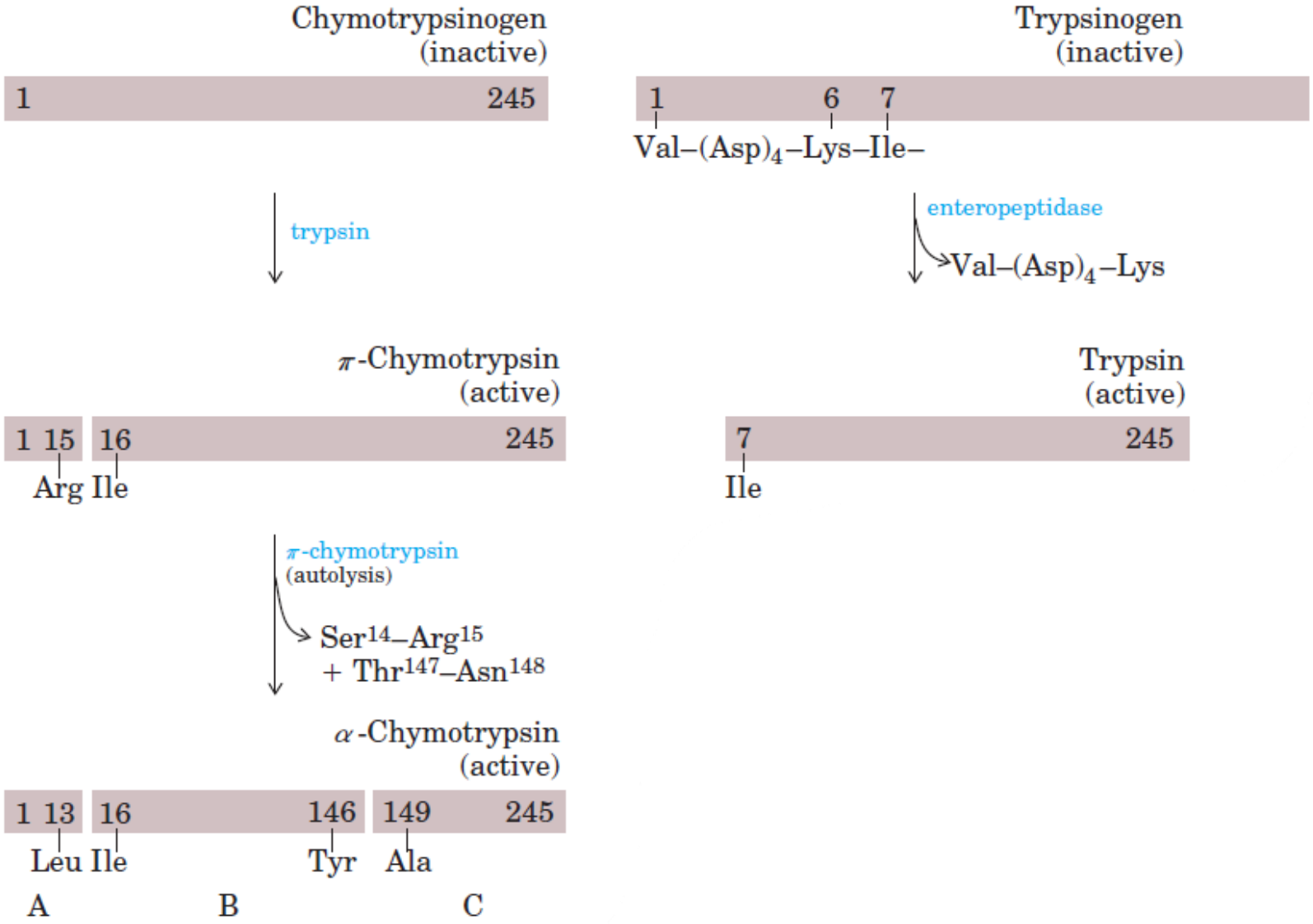
- Proteolytic enzymes are usually **biosynthesized as somewhat larger inactive precursors** known as **zymogens** (enzyme precursors, in general, are known as proenzymes).

- In the case of digestive enzymes, the reason for this is clear: If these enzymes were synthesized in their active forms, they would **digest the tissues that synthesized them**.

- Indeed, **acute pancreatitis**, a painful and sometimes fatal condition that can be precipitated by pancreatic trauma, is characterized by the **premature activation of the digestive enzymes** synthesized by that organ.

- The **activation of trypsinogen** (the **zymogen of trypsin**) occurs when trypsinogen enters the duodenum from the pancreas.

- **Enteropeptidase**, a serine protease whose secretion from the **duodenal mucosa** is **under hormonal control**, excises the N-terminal hexapeptide from trypsinogen by specifically cleaving its Lys5-Ile6 peptide bond.



The three polypeptide chains (A, B, and C) of chymotrypsin are linked by disulfide bonds

Zymogen Activation