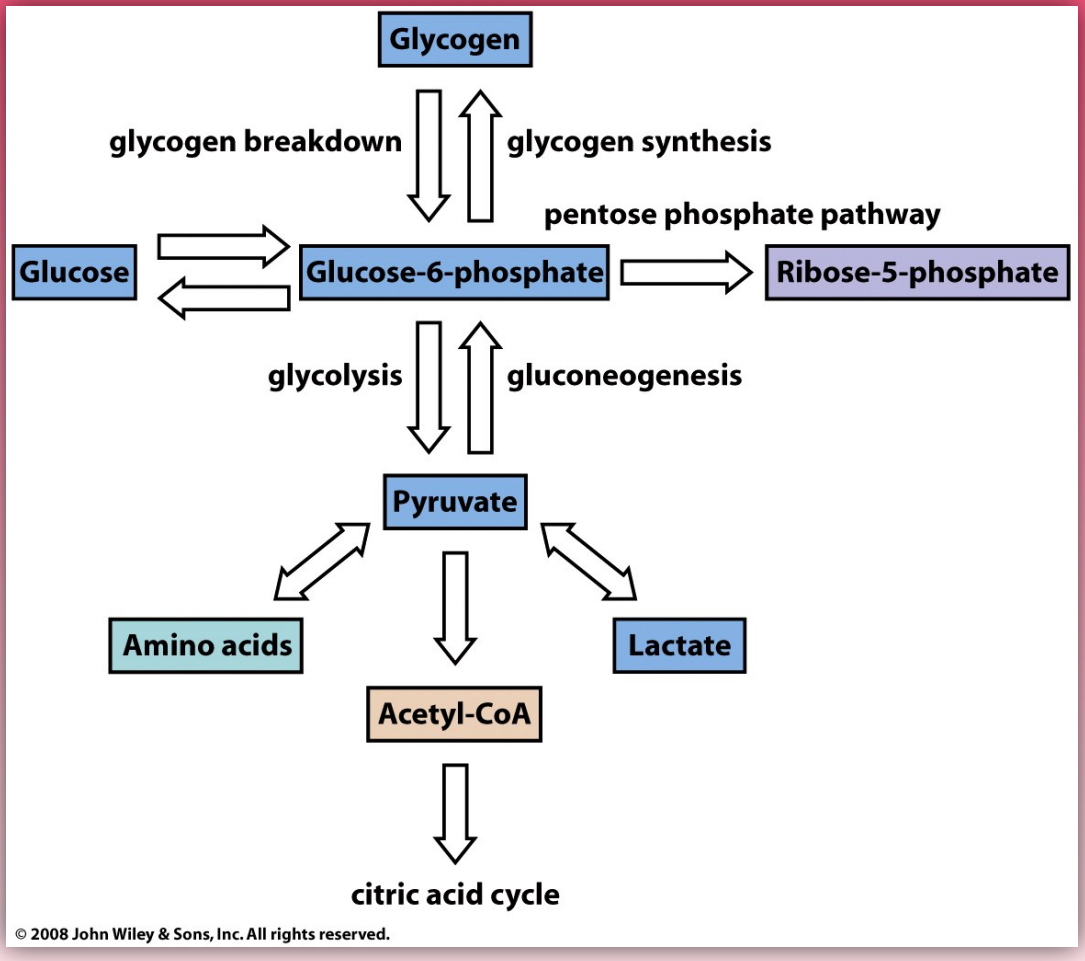
Glycogen Metabolism

CHEM 420 – Principles of Biochemistry
Instructor – Anthony S. Serianni

Chapter 18: Voet/Voet, *Biochemistry*, 2011
Fall 2015

November 16 & 18

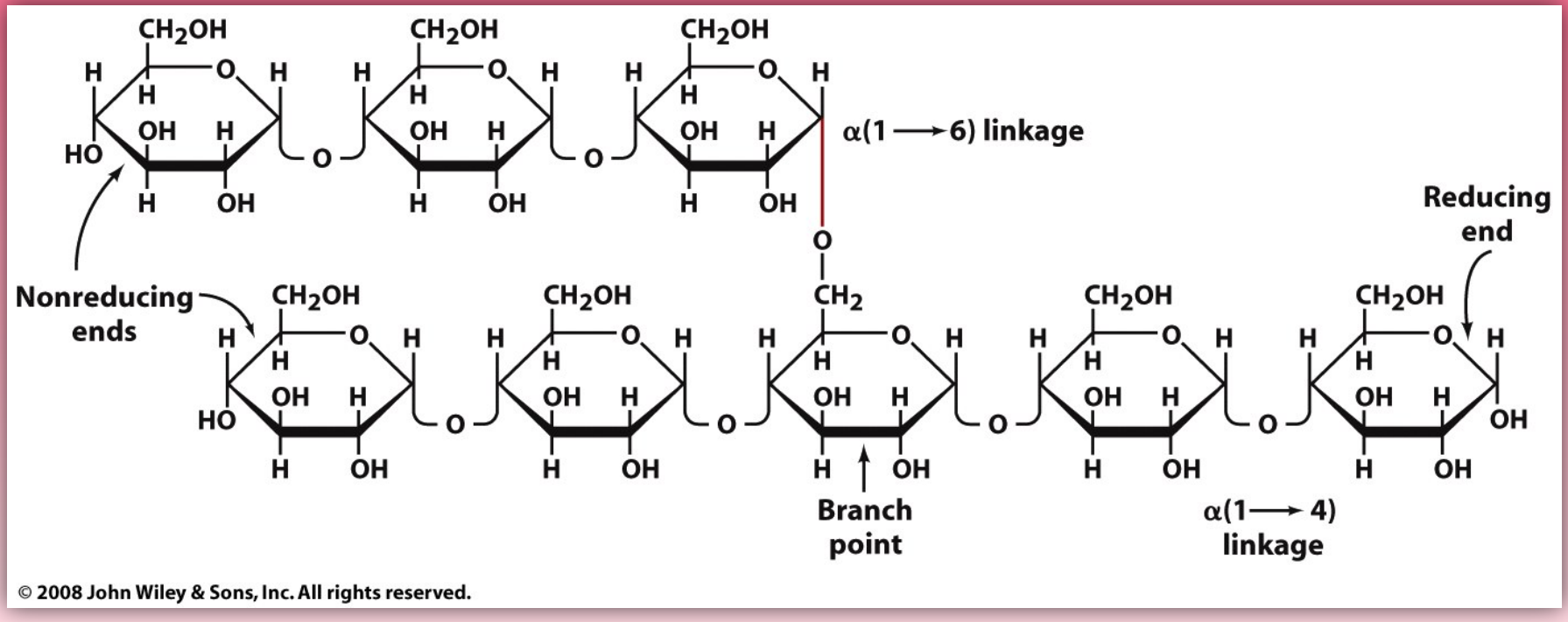


An overview of glucose metabolism

G6P occupies a cental position in glucose metabolism.

The G6P pool is supplied by glucose phosphorylation, *glycogen degradation*, and gluconeogenesis.

The structure of glycogen: α(1,4)-glucose polymer with α(1,6) linkages every 8-14 residues: resides in glycogen granules in the cytosol



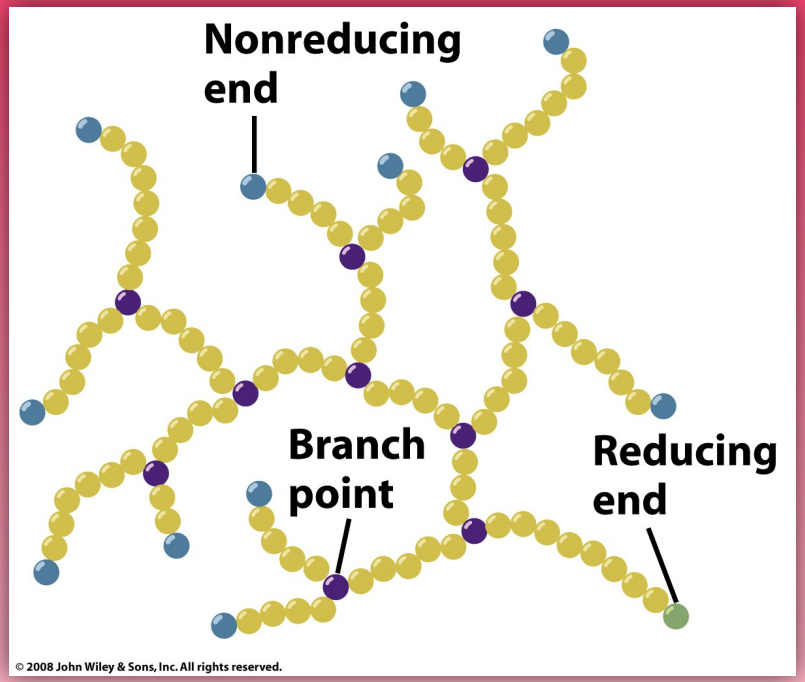
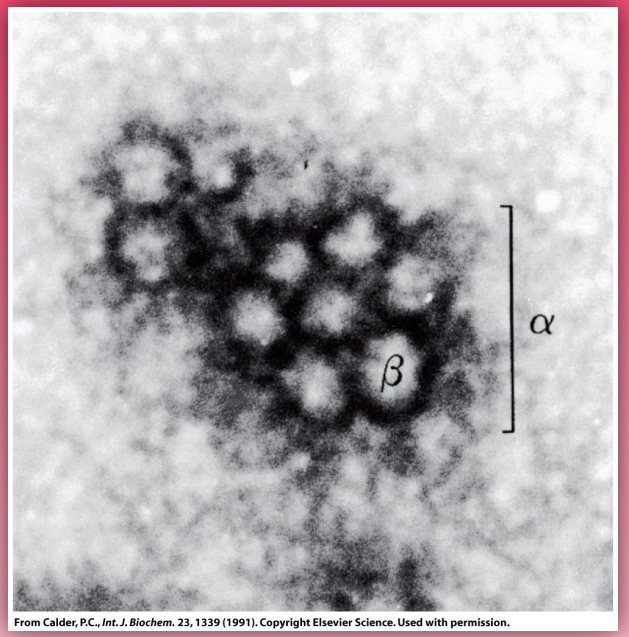


Diagram showing the branched structure of glycogen; the molecule has multiple non-reducing ends but only one reducing end.



Electron micrograph of a glycogen granule (α = granule; β = spherical glycogen molecules with associated proteins)

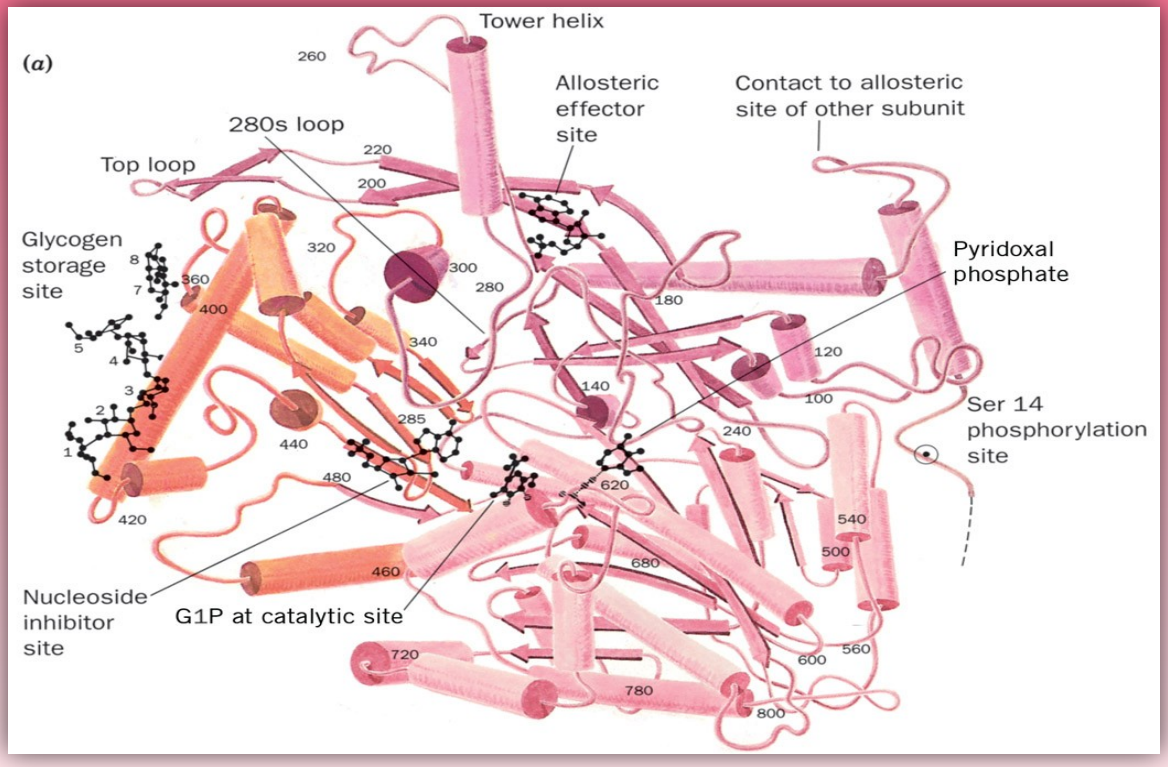
Three enzymes are responsible for glycogen breakdown in liver (*glycogenolysis*).

1. Glycogen phosphorylase: catalyzes *phosphorolysis* of glycosidic linkages (bond cleavage with the participation of P_i; G1P is the product

2. Glycogen debranching enzyme: removes glycogen branches

3. Phosphoglucomutase: converts G1P into G6P

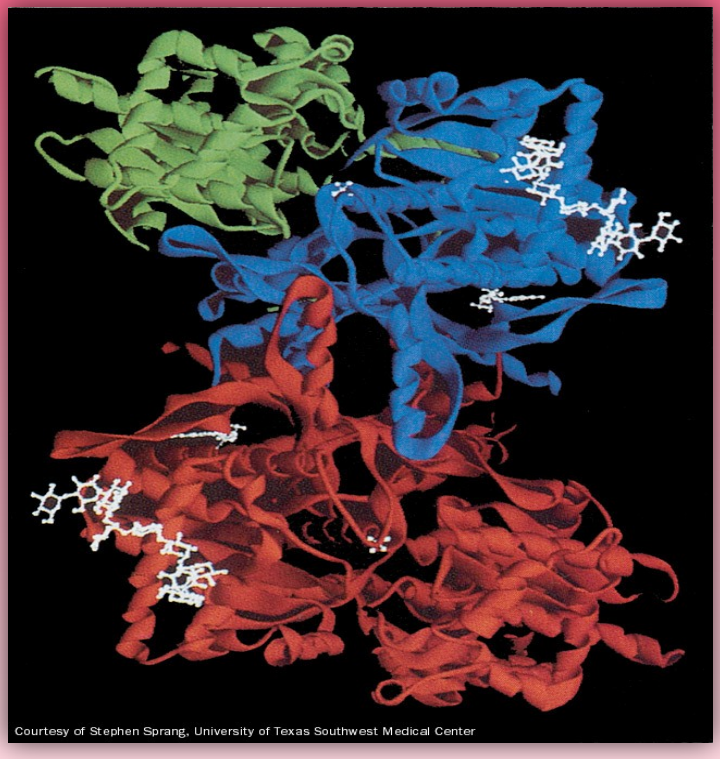
X-Ray structure of rabbit muscle glycogen phosphorylase: Ribbon diagram of a phosphorylase *b* subunit.



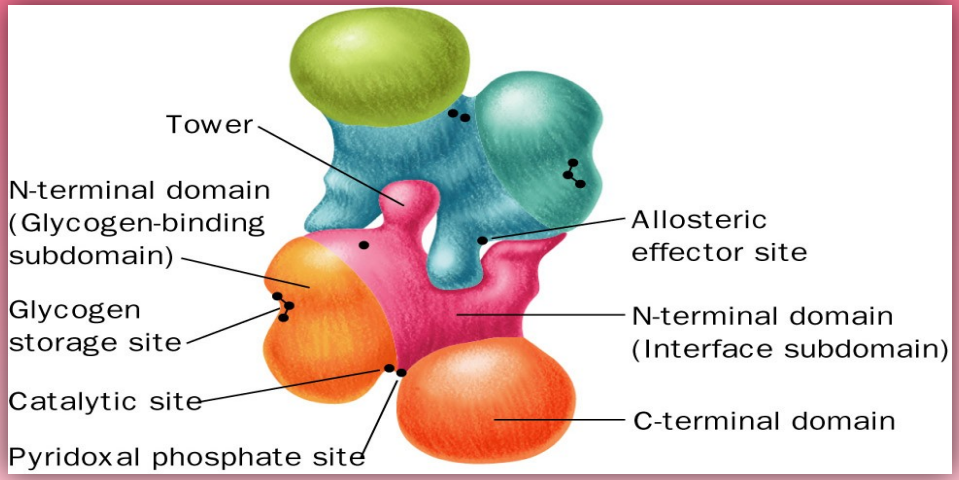
GP is a dimer of identical 842-residue (97 kD) subunits.

GP is regulated by allosteric interactions and by covalent modification. The enzyme exists in two states, phosphorylase *a* and phosphorylase *b*, with the former phosphorylated.

X-Ray structure of rabbit muscle glycogen phosphorylase

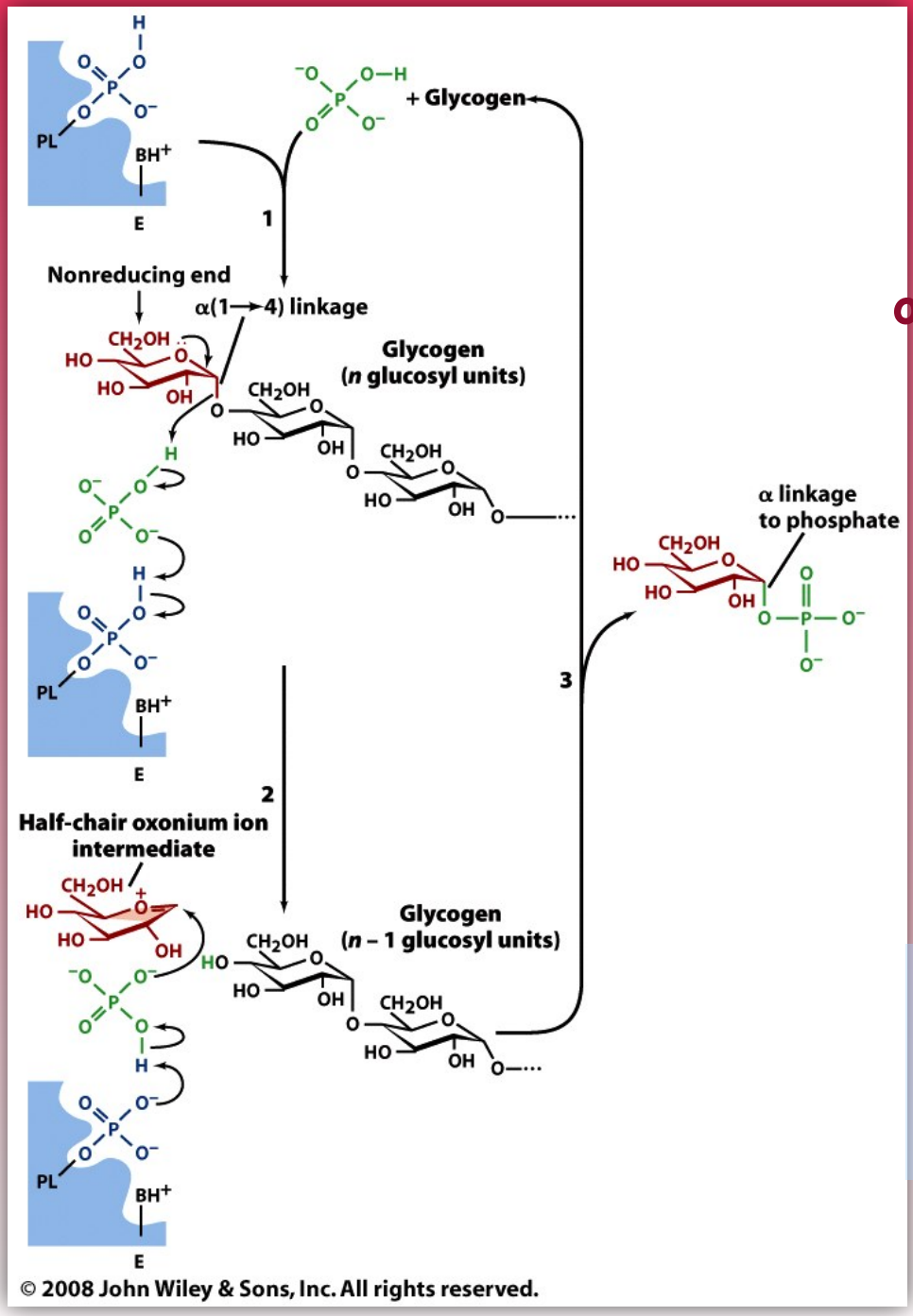


A ribbon diagram of the glycogen phosphorylase *a* dimer.

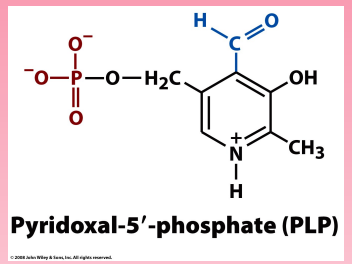


An interpretive "low-resolution" drawing of Part *b* showing the enzyme's various ligand-binding sites.

Reaction mechanism of glycogen phosphorylase



Contains PLP in its active site; serves as an acid-base catalyst



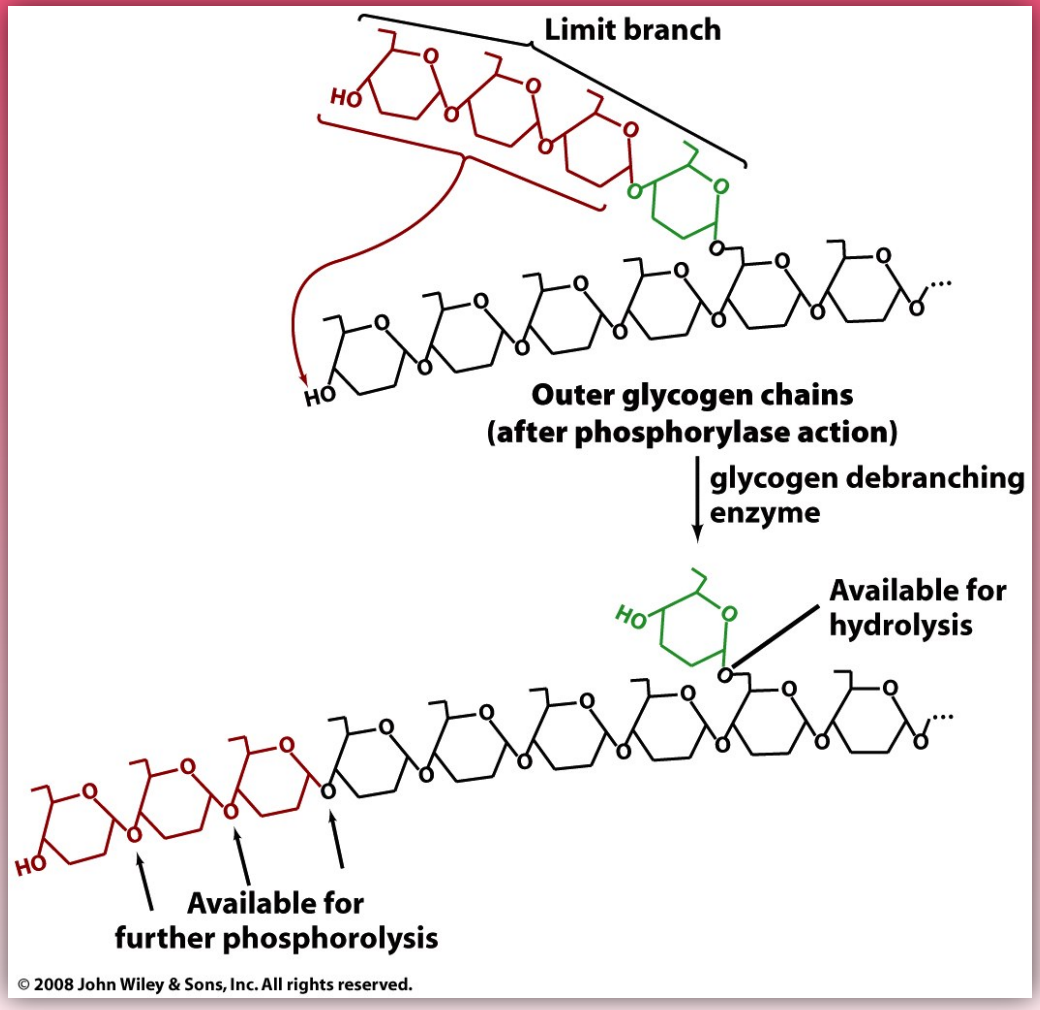
GP is regulated by allosteric interactions and by covalent modification (phosphorylation/ dephosphorylation).

Additional comments on the glycogen phosphorylase mechanism

The glycogen phosphorylase reaction proceeds with retention of configuration, suggesting the involvement of a covalent glucosyl-enzyme intermediate. However, the enzyme exhibits *Random Bi Bi* kinetics, not *Ping-Pong* kinetics, as would be expected for a double-displacement mechanism. Evidence for the existence of a covalent intermediate has not been found. For these reasons, a cyclic oxonium ion has been proposed as an intermediate in the reaction.

The active (R) and inactive (T) states of GP can be explained by gross changes in protein structure, with the T-state having a buried active site and the R-state having an accessible active site. Phosphorylation of the T-state activates the enzyme by facilitating conversion to the R-state. Alternatively, the unphosphorylated T-state can be activated allosterically by AMP to give an active, unphosphorylated R-form.

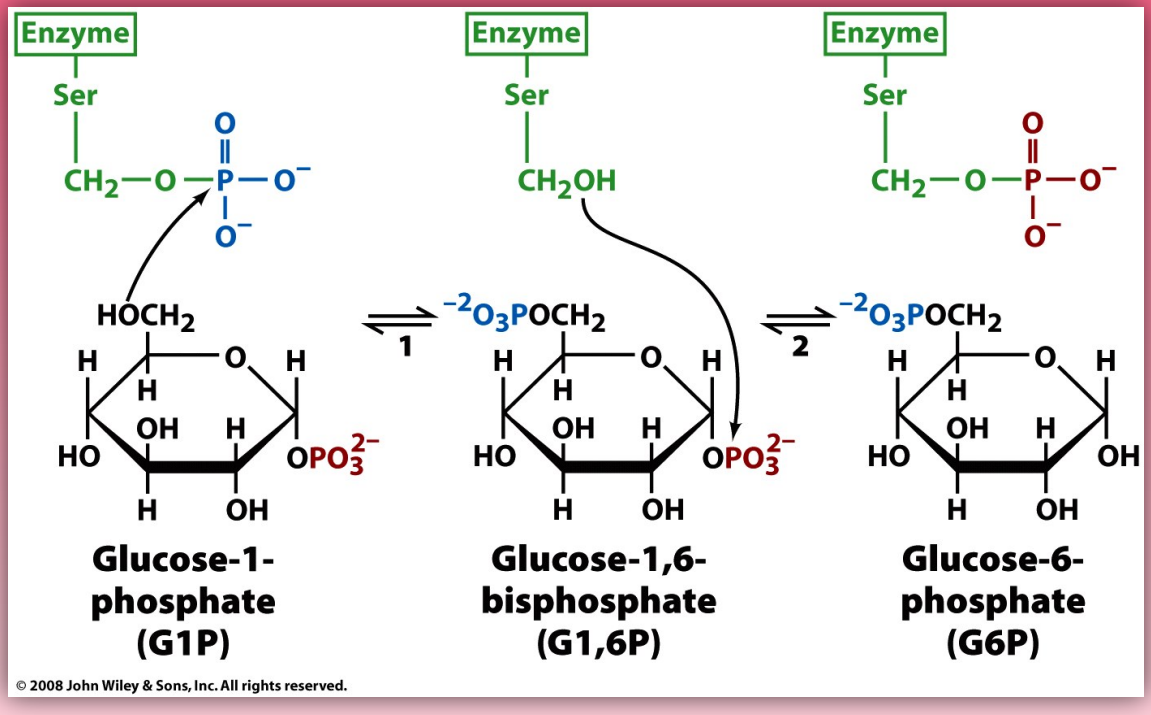
Reactions catalyzed by glycogen debranching enzyme



Acts as a transglycosylase and a glycosidase

GDE transfers the terminal three residues from a "limit branch" to the non-reducing end of another branch. The exposed $\alpha(1,6)$-linkage at the branch point is hydrolyzed to yield free glucose.

The mechanism of phosphoglucomutase



Mimics the mechanism of the glycolytic enzyme, phosphoglycerate mutase

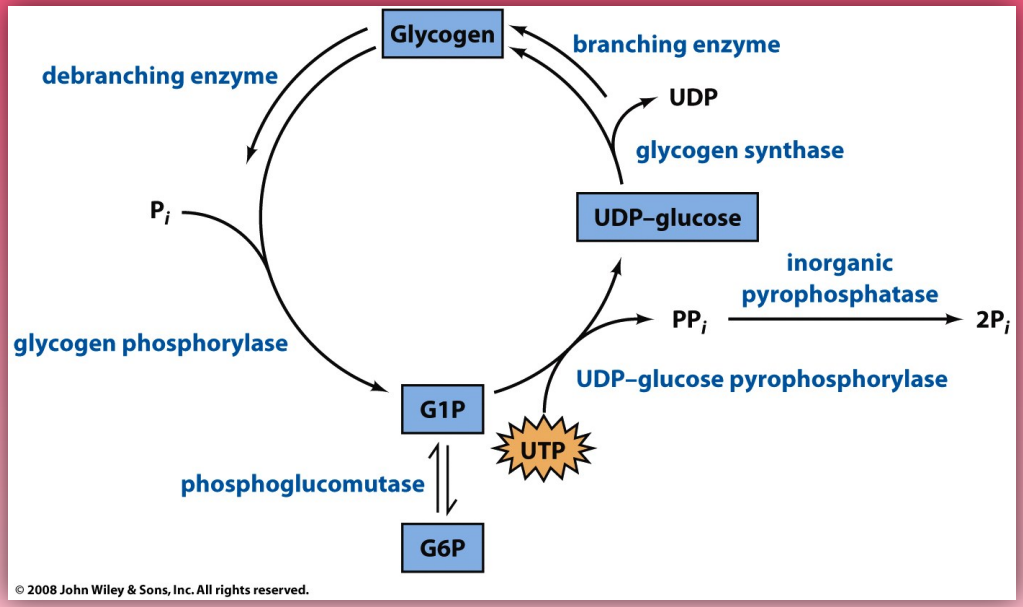
Examples of glycogen storage diseases in humans

Hereditary Glycogen Storage Diseases

Type	Enzyme Deficiency	Tissue	Common Name	Glycogen Structure
I	Glucose-6-phosphatase	Liver	von Gierke's disease	Normal
II	α-1,4-Glucosidase	All lysosomes	Pompe's disease	Normal
III	Amylo-1,6-glucosidase (debranching enzyme)	All organs	Cori's disease	Outer chains missing or very short
IV	Amylo-(1,4 → 1,6)-transglycosylase (branching enzyme)	Liver, probably all organs	Andersen's disease	Very long unbranched chains
V	Glycogen phosphorylase	Muscle	McArdle's disease	Normal
VI	Glycogen phosphorylase	Liver	Hers' disease	Normal
VII	Phosphofructokinase	Muscle	Tarui's disease	Normal
VIII	Phosphorylase kinase	Liver	X-Linked phosphorylase kinase deficiency	Normal
IX	Phosphorylase kinase	All organs		Normal
0	Glycogen synthase	Liver		Normal, deficient in quantity

Inherited disorders that affect glycogen metabolism

The opposing pathways of glycogen synthesis and degradation



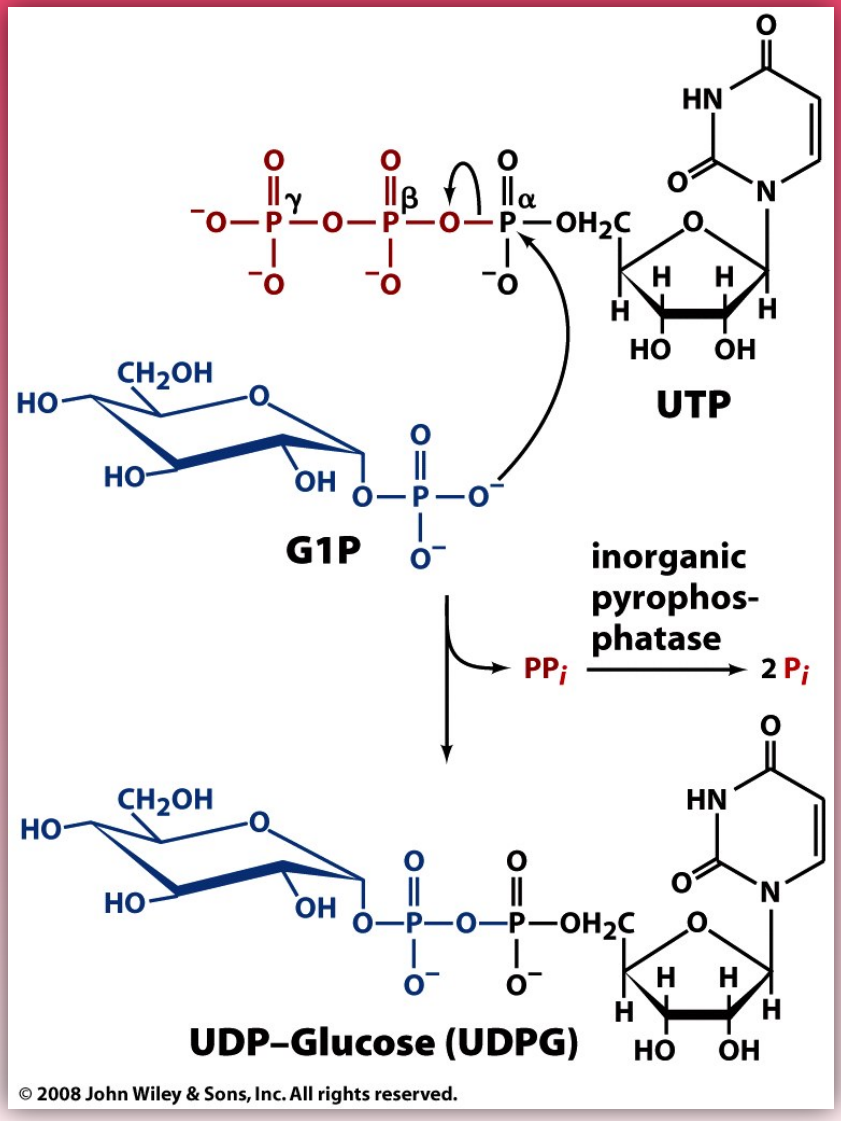
$\Delta G^{o\prime}$ (glycogen phosphorylase) = +3.1 kJ/mol, but under physiological conditions, the overall ΔG for glycogen breakdown is -5 to -8 kJ/mol: glycogen degradation is thermodynamically favored.

Glycogen biosynthesis is driven by the conversion of G1P into the "biologically-activated sugar", UDP-glucose, which serves as the sugar donor, with subsequent release and hydrolysis of PP_i driving the reaction.

Glycogen biosynthesis enzymes

1. Glycogen synthase (allosteric): enzyme responsible for constructing the α(1,4)-glycosidic linkages of glycogen using UDP-glucose as the sugar donor

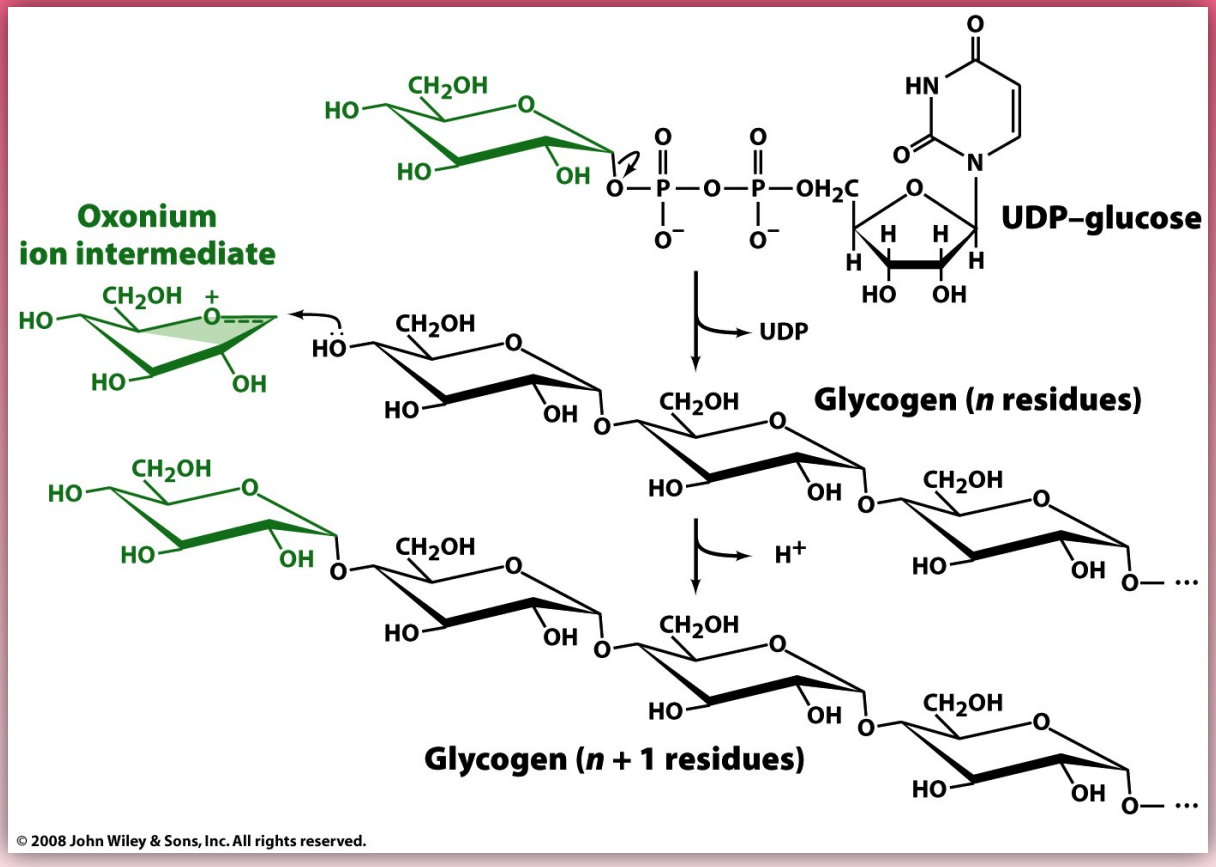
2. Glycogen branching enzyme: enzyme responsible for introducing α(1,6)-glycosidic linkages into glycogen (branching)



The reaction catalyzed by UDP-glucose pyrophosphorylase: formation of UDP-glucose

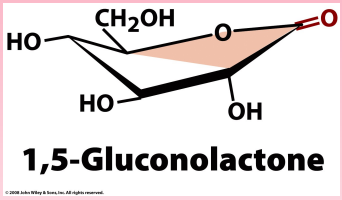
$\Delta G^{o\prime}$ for this reaction is approximately zero (a phosphoanhydride exchange reaction), but subsequent PP_i hydrolysis drives the reaction to completion.

The reaction catalyzed by glycogen synthase



GS (human muscle) is a homotetramer of 737-residue subunits. The reaction proceeds with retention of configuration and involves an oxonium ion intermediate.

$\Delta G^{o\prime}$ for glycogen biosynthesis is -13.4 kJ/mol.



D-Glucono-1,5-lactone is a potent inhibitor of GS.

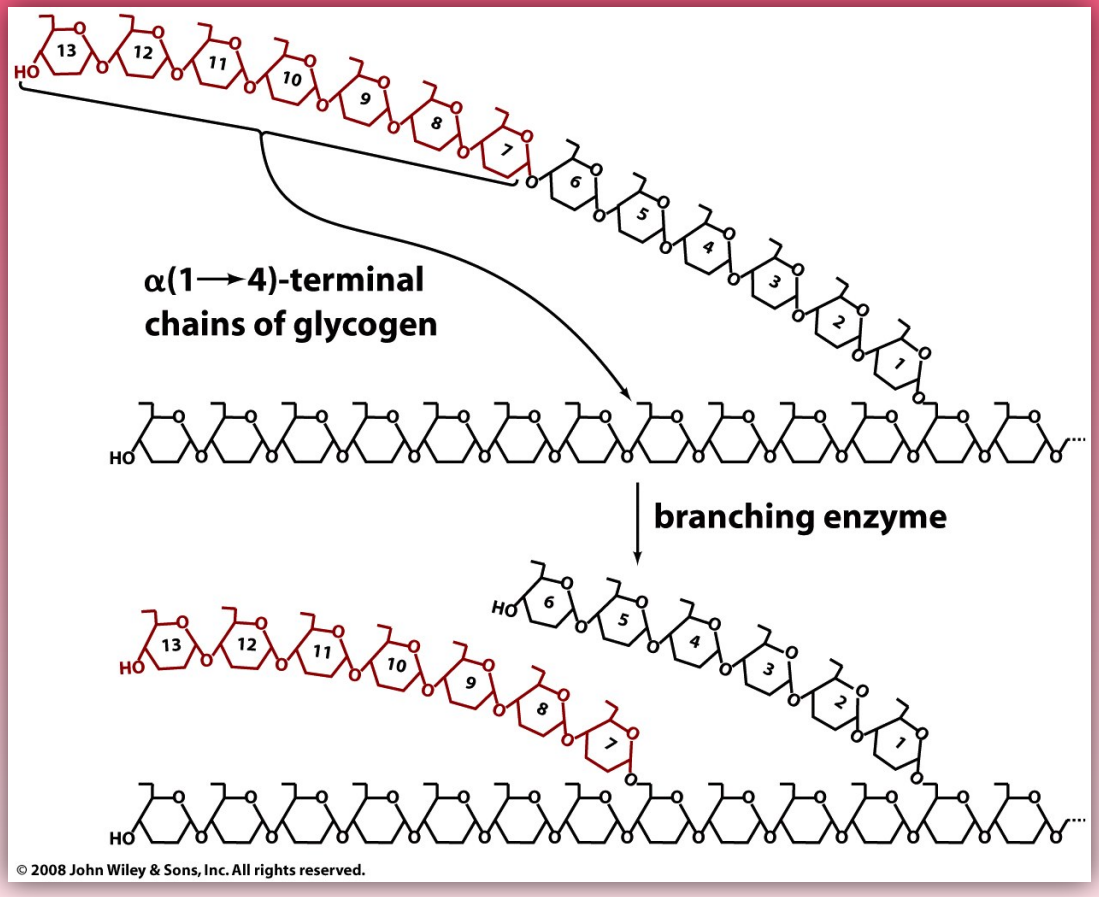
Additional information on glycogen synthase

GS is an allosteric enzyme. Its activity is modulated by allosteric effectors and by covalent modification. The latter involves phosphorylation/ dephosphorylation; in this case, phosphorylation of GS (*b* form) *inactivates* the enzyme, and the dephospho form (*a* form) is active (opposite to what is observed for glycogen phosphorylase).

Comment on the mechanisms of GP and GS

Both involve an oxonium ion intermediate. In this sense, both GP and GS resemble the glycosidase, *lysozyme*, that hydrolyzes the glycosidic linkages of the bacterial cell wall peptidoglycan.

Action of glycogen branching enzyme



Branches are formed by transferring a 7-residue terminal segment from an α(1,4)-linked glucan chain to the C6 oxygen of a glucose residue on the same chain or another chain.

The branching reaction is favored energetically: $\Delta G^{o\prime}$ of hydrolysis of α(1,4)-linkages = -15.5 kJ/mol; $\Delta G^{o\prime}$ of hydrolysis of α(1,6)-linkages = -7.1 kJ/mol.

Initiation of glycogen synthesis

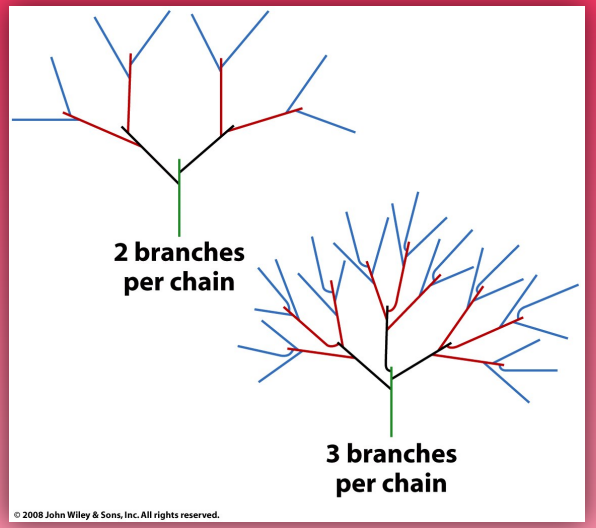
Glycogen synthase cannot link two glucose monomers together; it can only extend an existing α(1,4) chain.

Glycogen does not have a "reducing end". The single "reducing-end" glucose residue is covalently attached to a 349-residue dimeric protein, glycogenin.

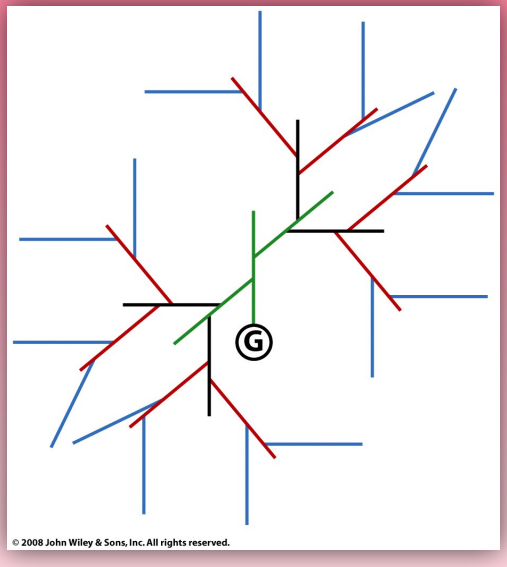
Glycogenin is a glycosyltransferase. The enzyme attaches a glucose residue from UDP-glucose to its free OH group of Tyr 194 (autocatalytic). It then extends the glucose chain to a 7-8 mer, creating a glycogen "primer". Glycogen synthase then acts on this primer.

Optimal structure of glycogen

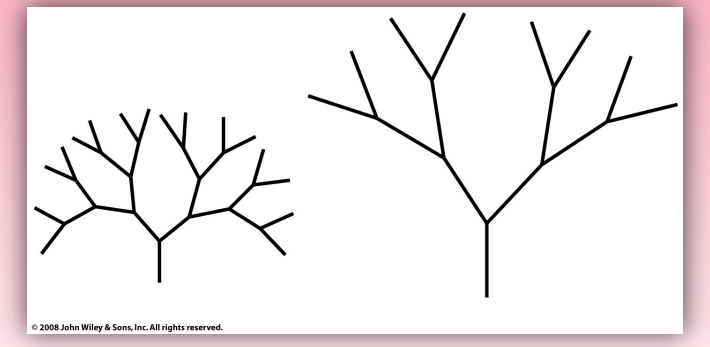
Maximal glucose storage in minimal volume; maximal rate of mobilization



With three branches per chain, the maximal size of the glycogen particle is limited.



Glycogen with two branches per chain; *in vivo* state



Optimal chain length: 8-14 residues

Metabolic control of glycogen synthesis and degradation: Part B

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Chapter X: Voet/Voet, *Biochemistry*, 2011
Spring 2015

Enzymes involved in covalent modification of glycogen phosphorylase

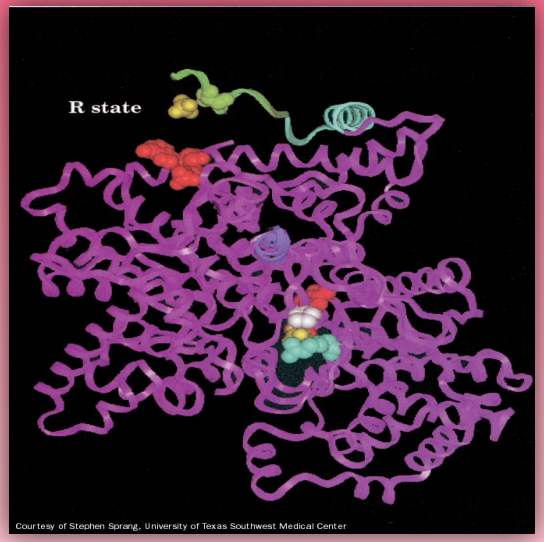
1. Glycogen phosphorylase kinase: specifically phosphorylates Ser 14 of glycogen phosphorylase *b*; its activity is modified by phosphorylation and by Ca^{2+}; a 1300 kD protein with four non-identical subunits (α, β, γ and δ) (γ is the catalytic subunit; δ is the calcium-binding subunit, calmodulin)

2. Protein kinase A (PKA): phosphorylates and thus activates phosphorylase kinase; PKA is activated by cAMP

3. Phosphoprotein phosphatase-1 (PP1): dephosphorylates and thus deactivates glycogen phosphorylase *a* and phosphorylase kinase

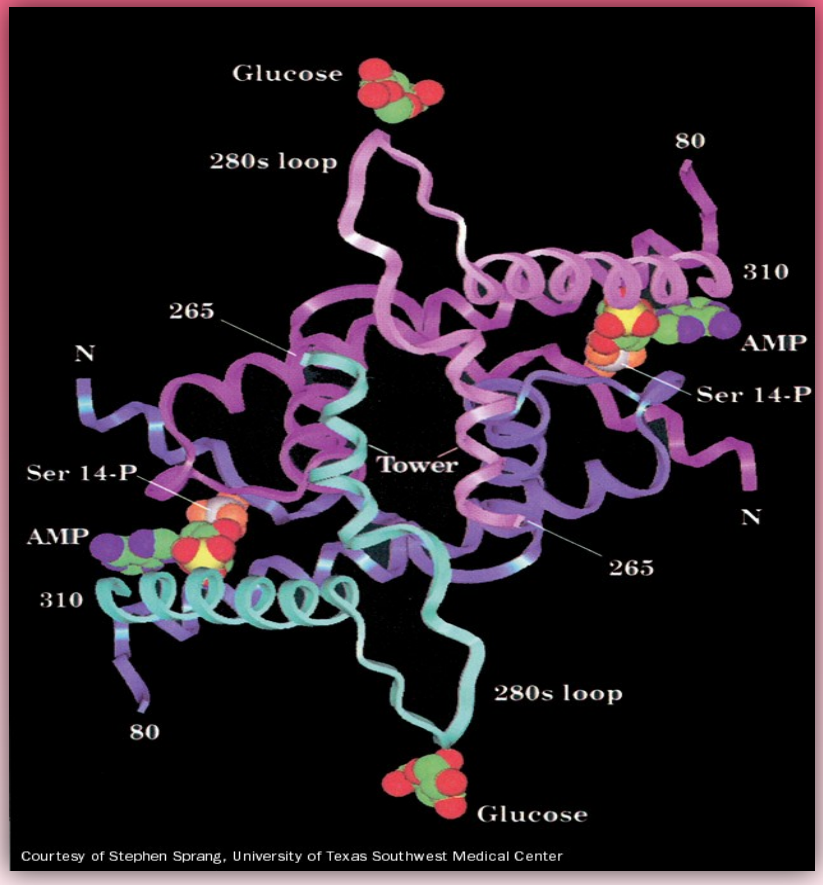
Conformational changes in glycogen phosphorylase

Ribbon diagram of one subunit (T-state) in the absence of allosteric effectors

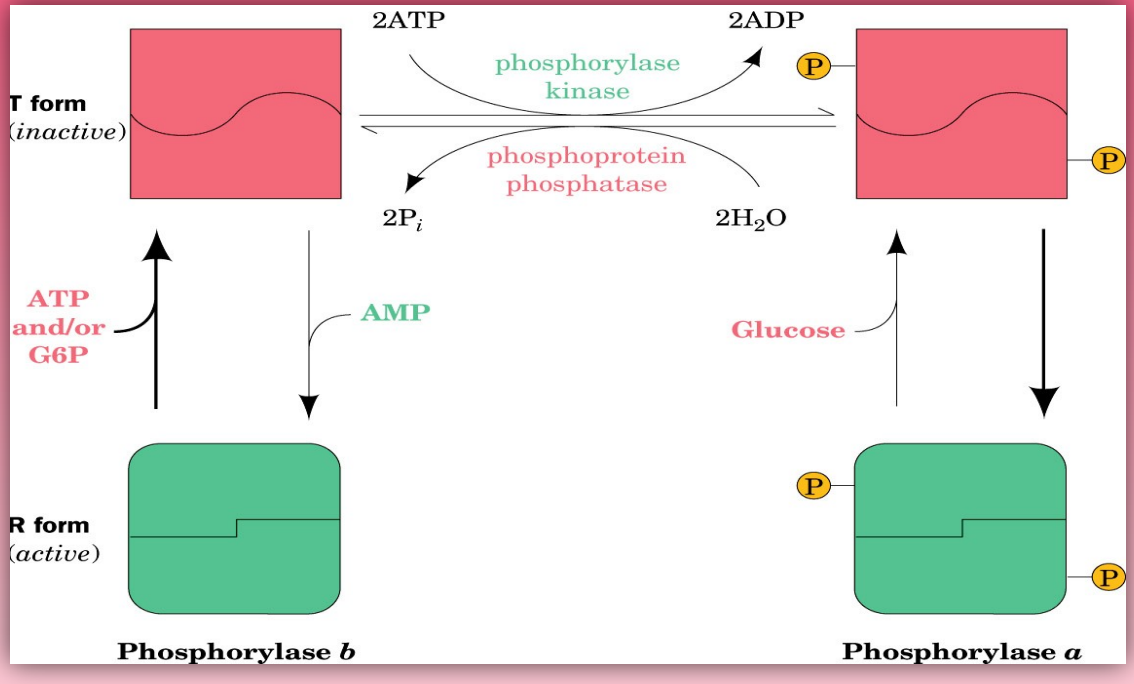


Ribbon diagram of one subunit (R-state) with bound AMP

Conformational changes in glycogen phosphorylase: The portion of the glycogen phosphorylase *a* dimer in the vicinity of the dimer interface.



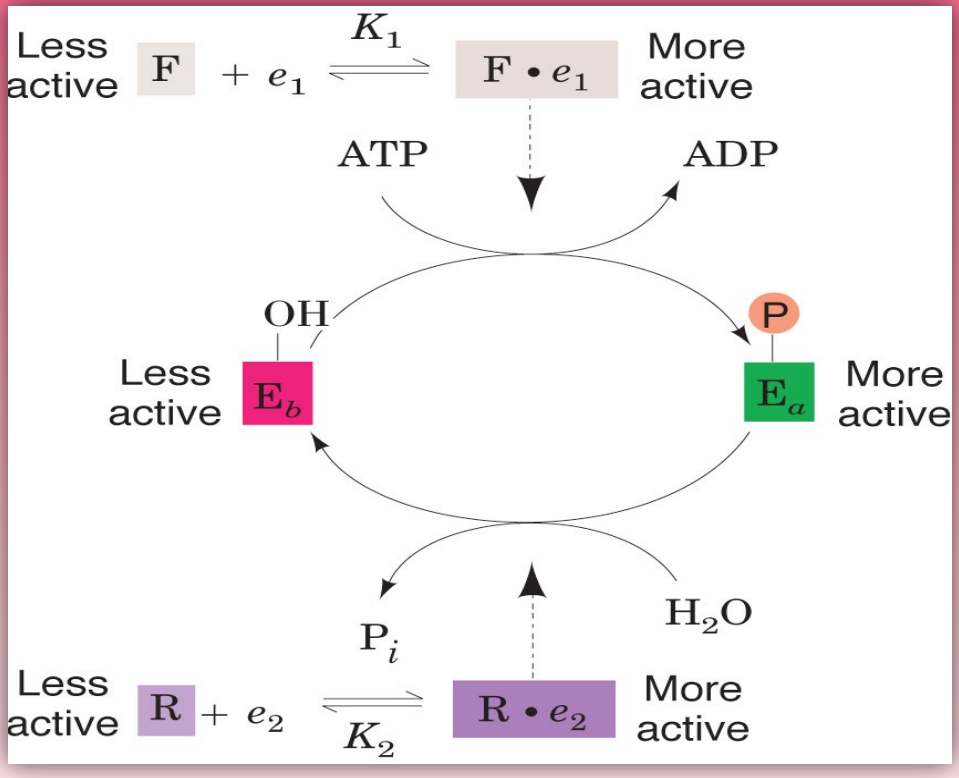
Control of glycogen phosphorylase activity



GP activity is controlled allosterically and covalently.

Only glycogen phosphorylase *b* binds the allosteric effector, AMP, thus converting it into an active enzyme (the *b* form is AMP-dependent).

Different forms of glycogen phosphorylase and glycogen synthase are interconverted through *cyclic cascades*

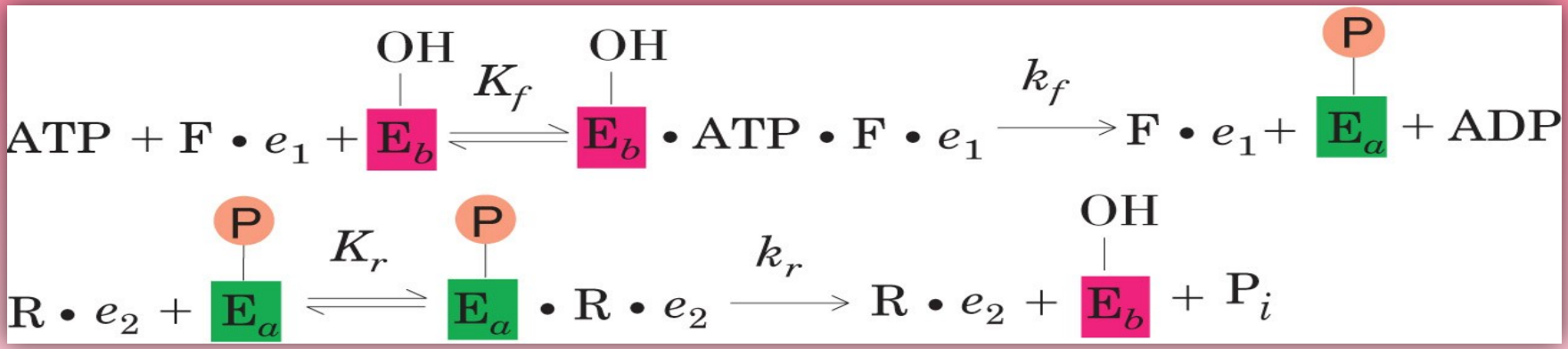


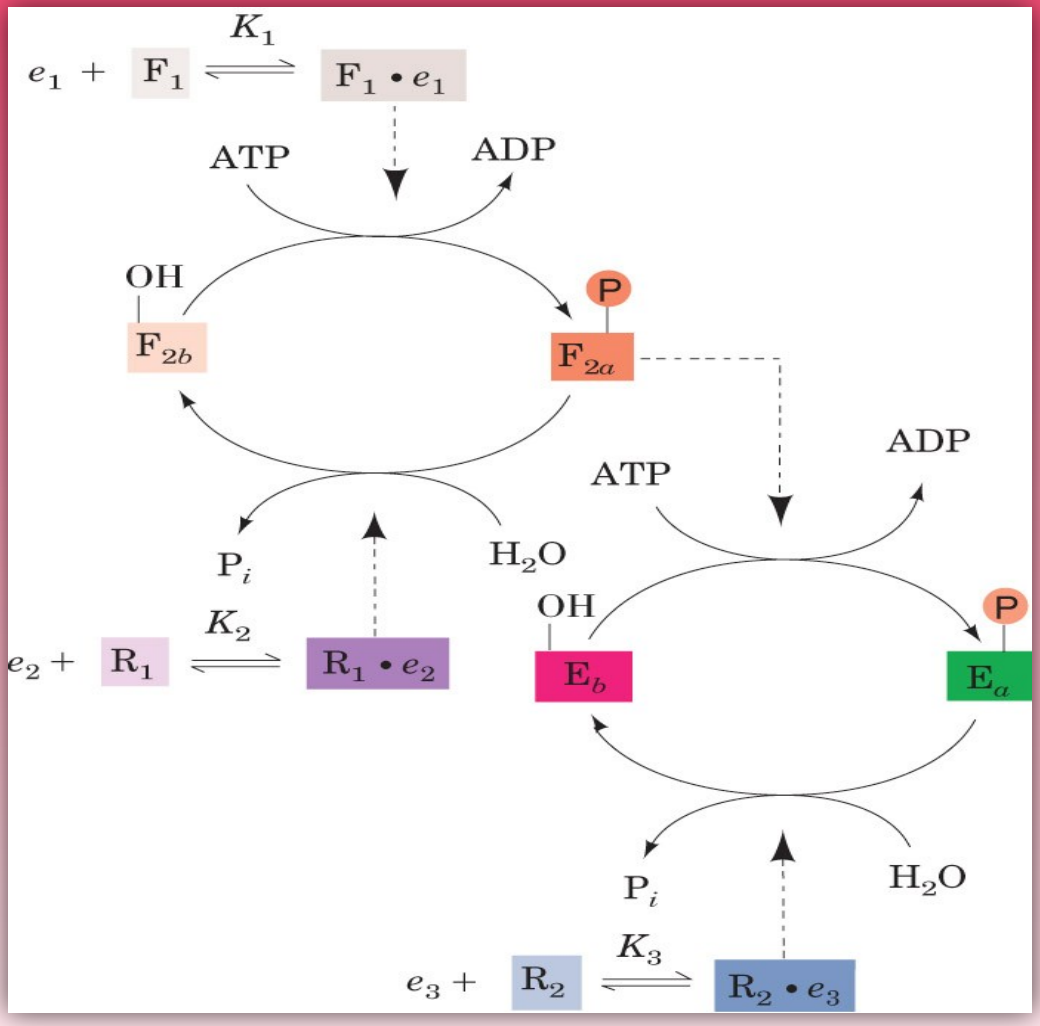
A monocyclic enzyme cascade; a general scheme, where F and R are, respectively, the modifying and demodifying enzymes, and e_1 and e_2 are effectors.

Enzyme E is the enzyme whose activity is controlled via

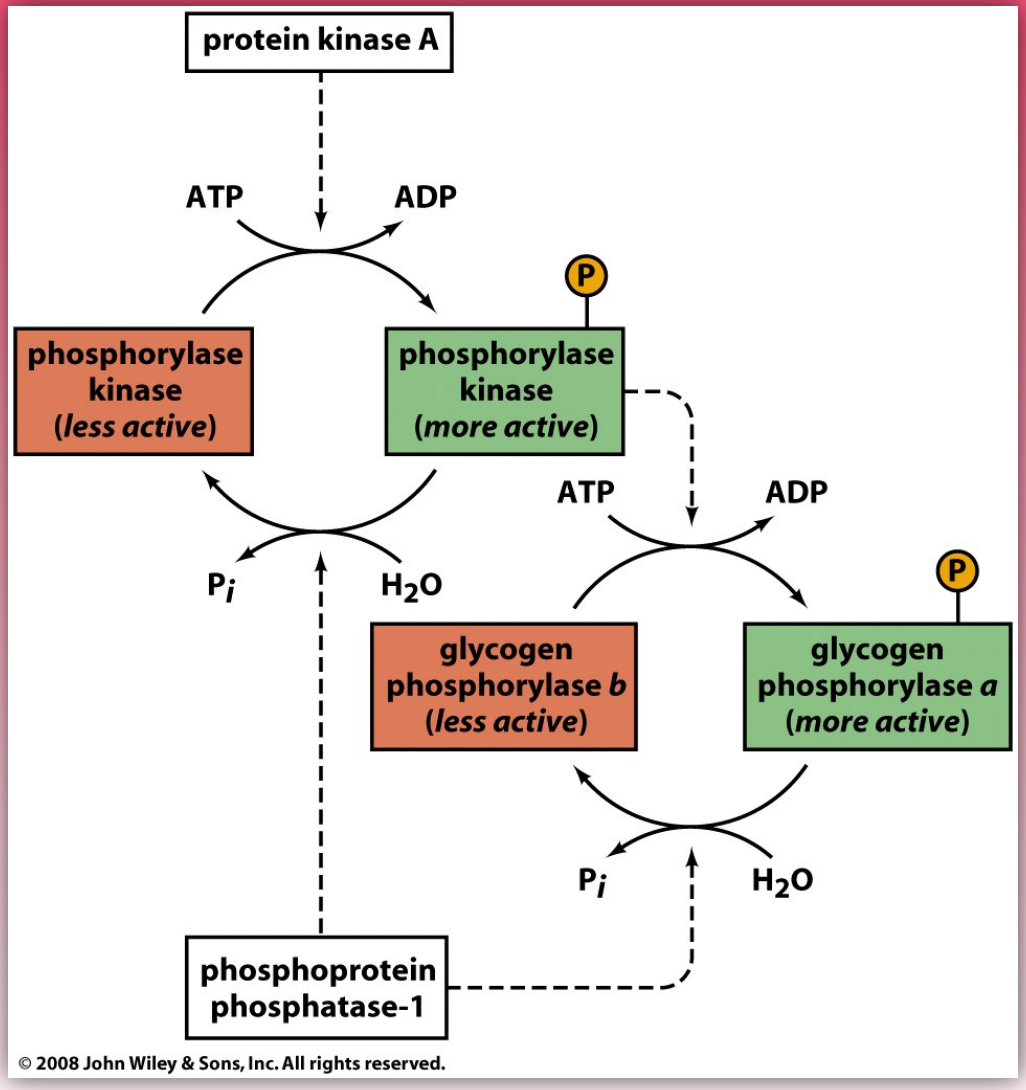
phosphorylation.

A generalized scheme for *monocyclic* enzyme cascade: Chemical equations for the interconversion of the target enzyme's unmodified and modified forms, E_b and E_a





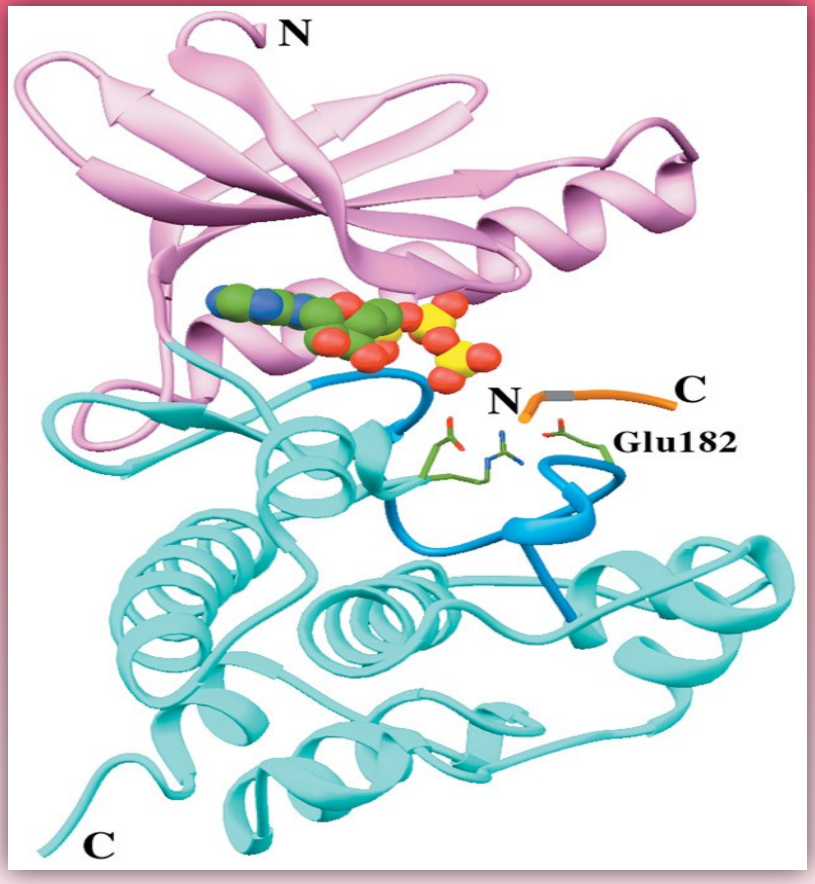
A generalized example of a bicyclic enzyme cascade



The glycogen phosphorylase cascade system of activation/ deactivation (a bicylic cascade)

The conversion of GP*b* to GP*a* is achieved via phosphorylation by GP kinase, which is activated by protein kinase A (PKA), which is in turn activated by cAMP. GP*a* and GP kinase are deactivated by the same phosphatase, PP-1

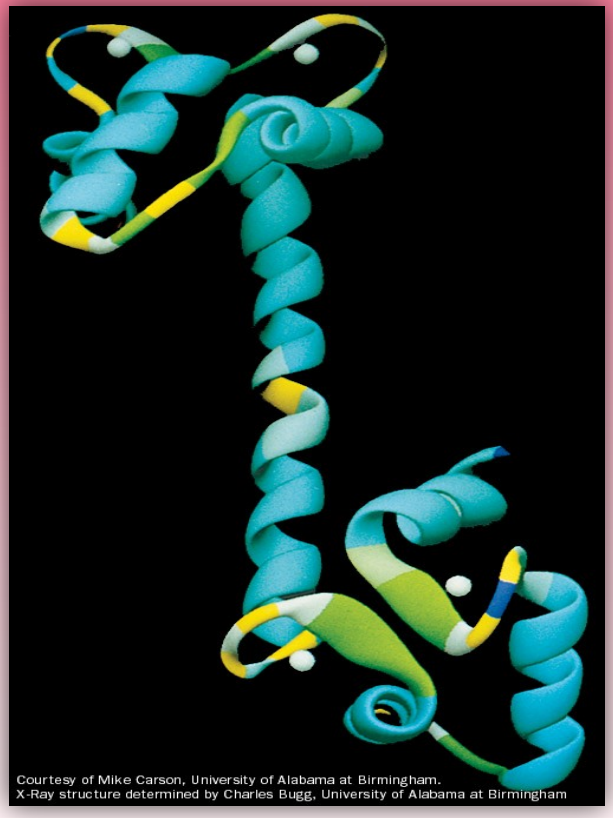
X-ray structure of rabbit muscle GP kinase in complex with ATP and a substrate analog heptapeptide, RQMSFRL



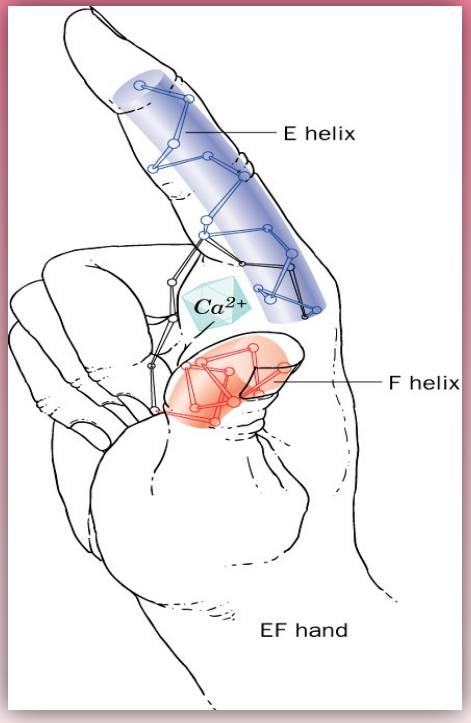
N-terminal domain in pink; C-terminal domain in cyan; activation loop in light blue; heptapeptide in orange, with serine residue in white.

GP kinase is maximally activated by binding Ca^{2+} ion (δ subunit is calmodulin, CaM)

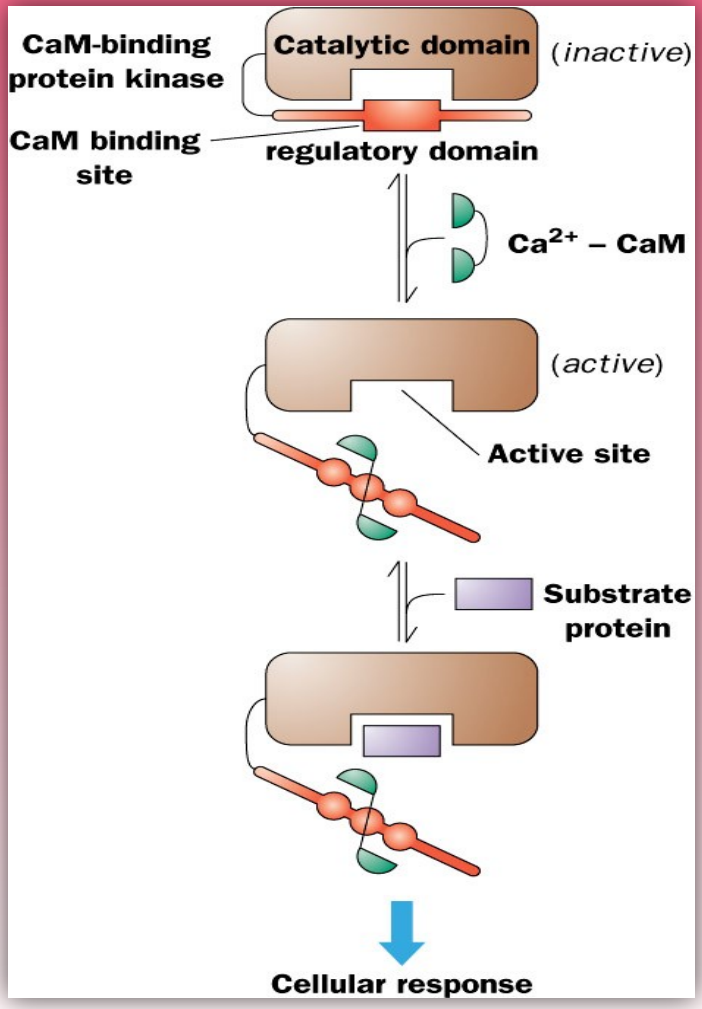
X-Ray structure of rat testis calmodulin



EF hand: The Ca^{2+} binding sites in many proteins that function to sense the level of Ca^{2+} are formed by helix-loop-helix motifs called EF hands.

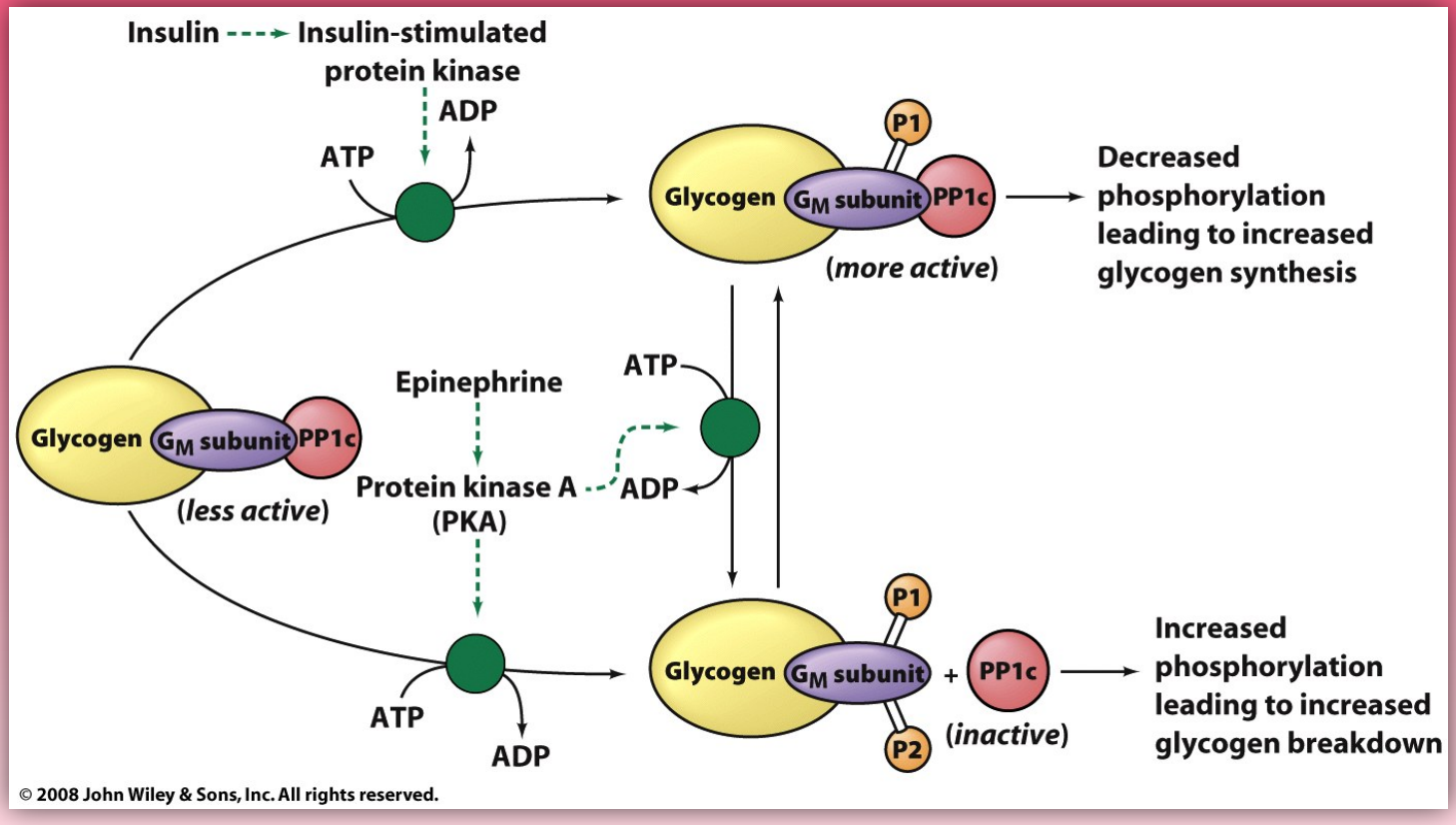


Schematic diagram of the Ca^{2+}–CaM-dependent activation of protein kinases



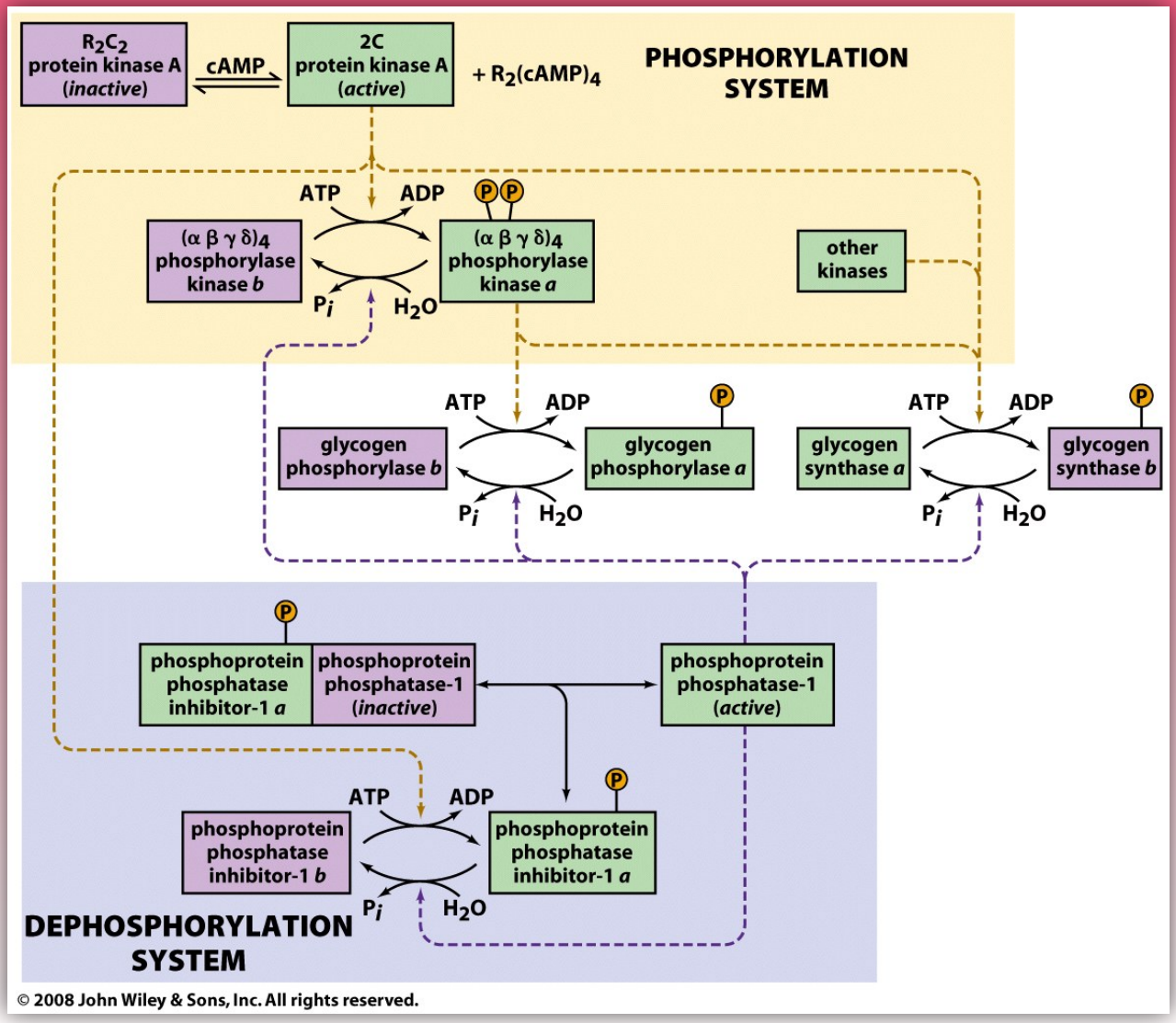
Autoinhibited kinases possess an *N*-terminal or *C*-terminal "pseudosubstrate" that is displaced by Ca^{2+}-CaM.

The antagonistic effects of insulin (high glucose) and epinephrine (stress) on glycogen metabolism in muscle



Occur through their effects on the PP-1 catalytic subunit, PP1c, via its glycogen-bound G_M subunut.

Schematic diagram of the major enzymatic modification/demodification systems involved in the control of glycogen metabolism in muscle



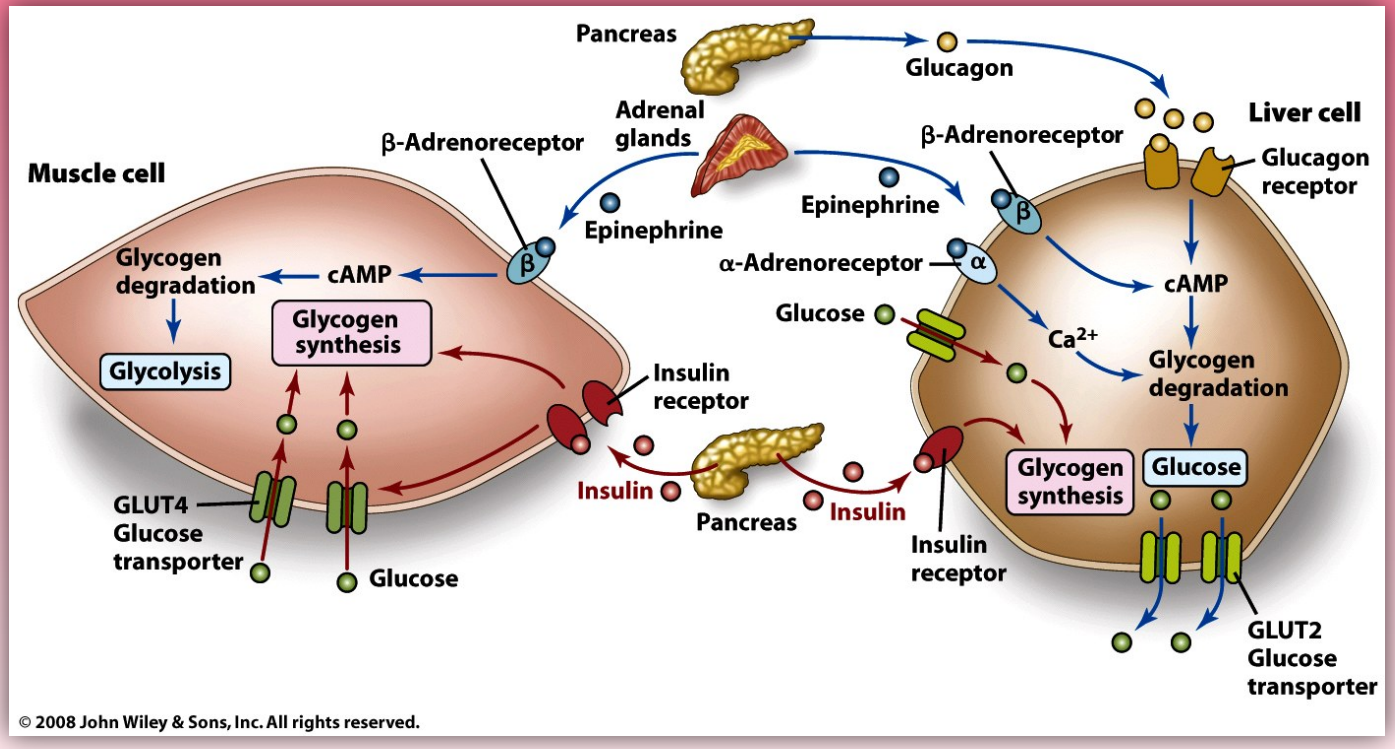
$H_3\overset{+}{N}$—His—Ser—Glu—Gly—Thr—Phe—Thr—Ser—Asp—Tyr—10

Ser—Lys—Tyr—Leu—Asp—Ser—Arg—Arg—Ala—Gln—20

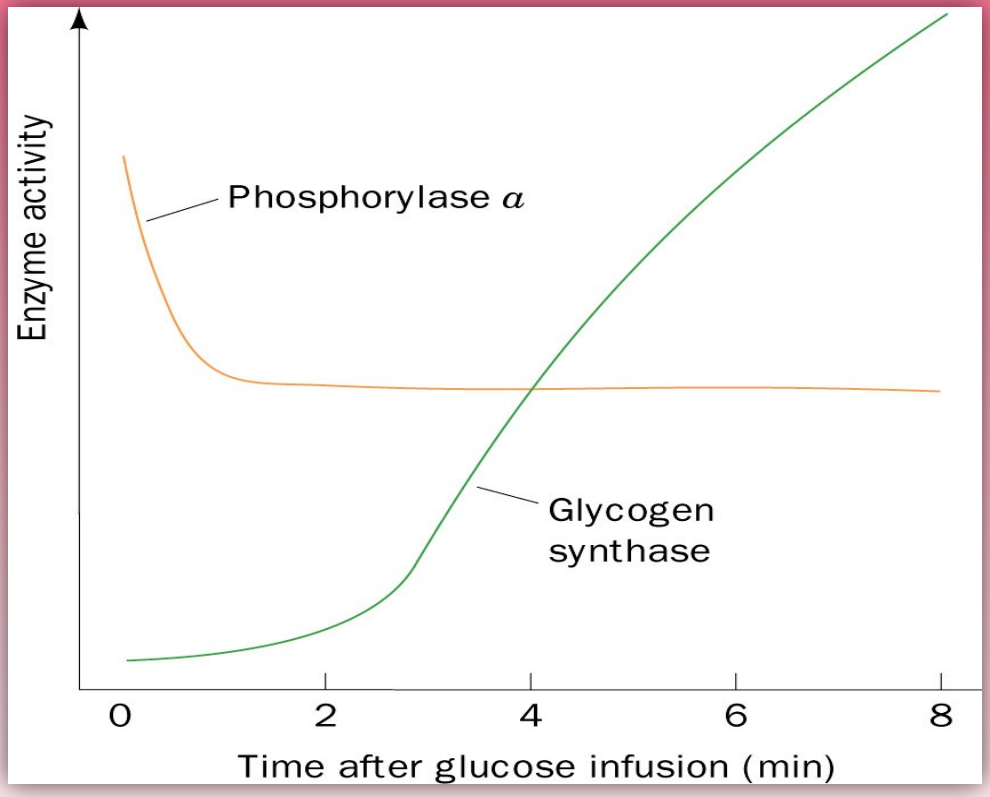
Asp—Phe—Val—Gln—Trp—Leu—Met—Asn—Thr—COO^- 29

Glucagon

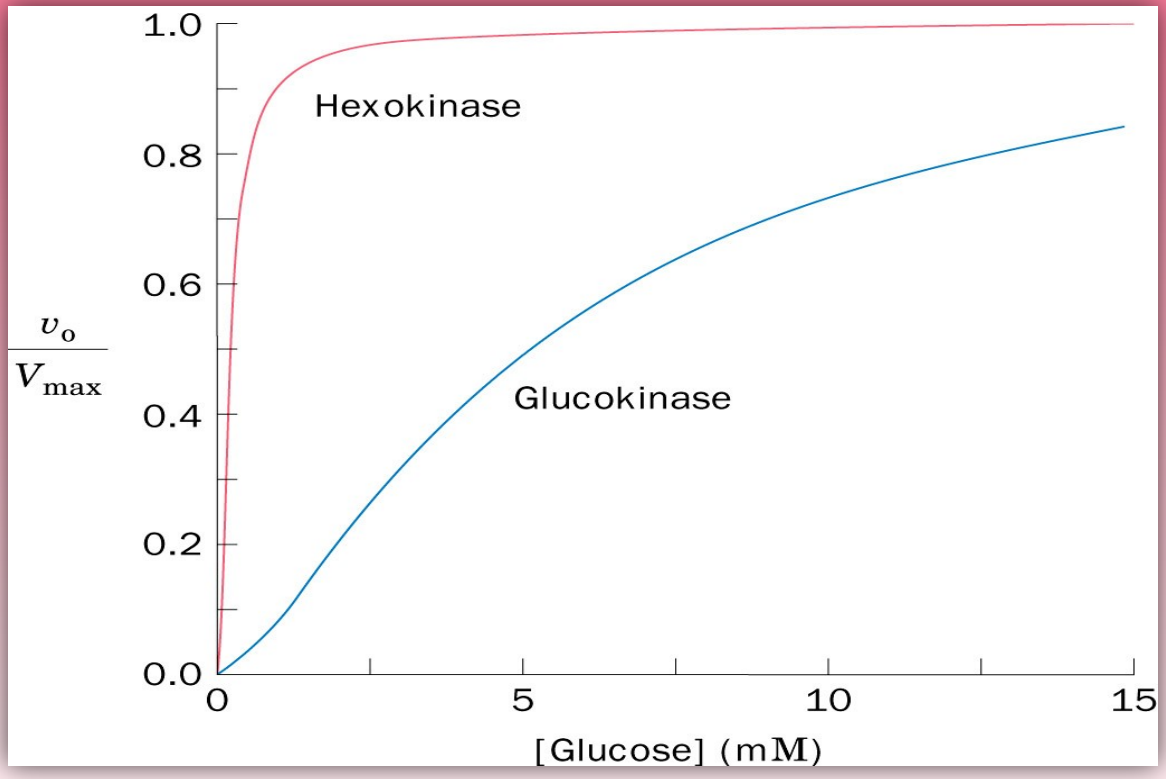
Glycogen metabolism is subject to hormonal control.



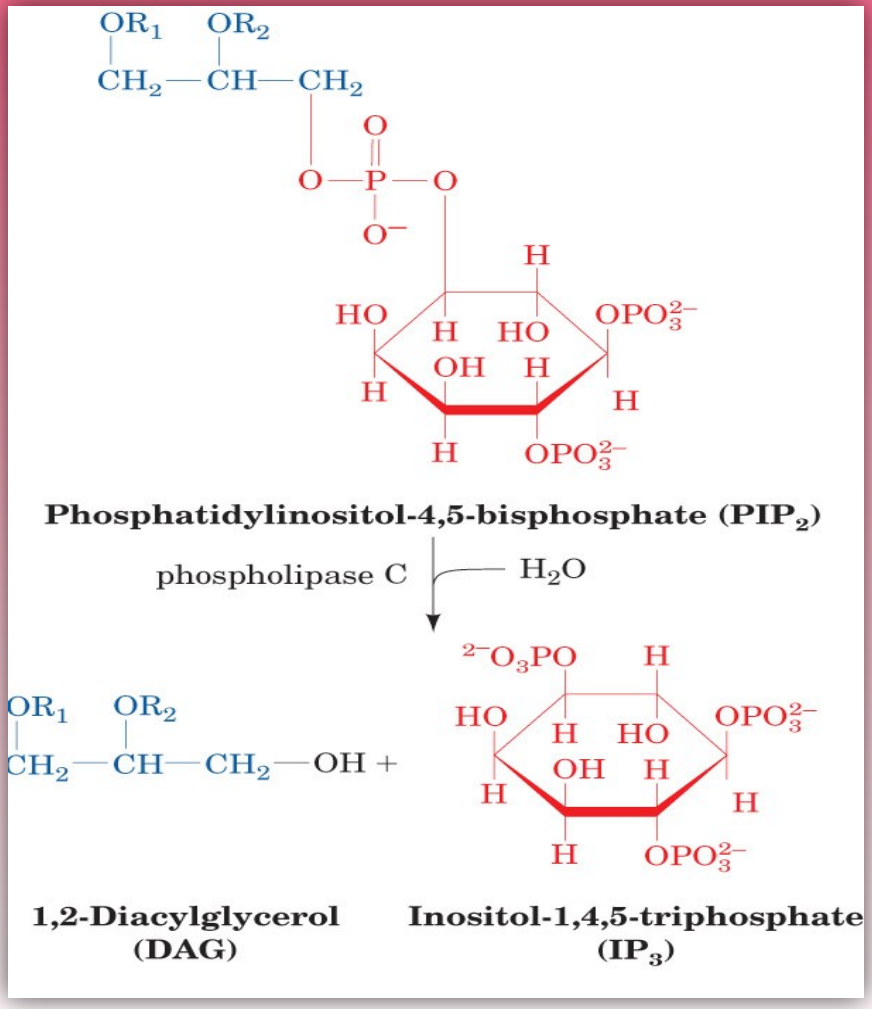
The enzymatic activities of glycogen phosphorylase *a* and glycogen synthase in mouse liver in response to an infusion of glucose



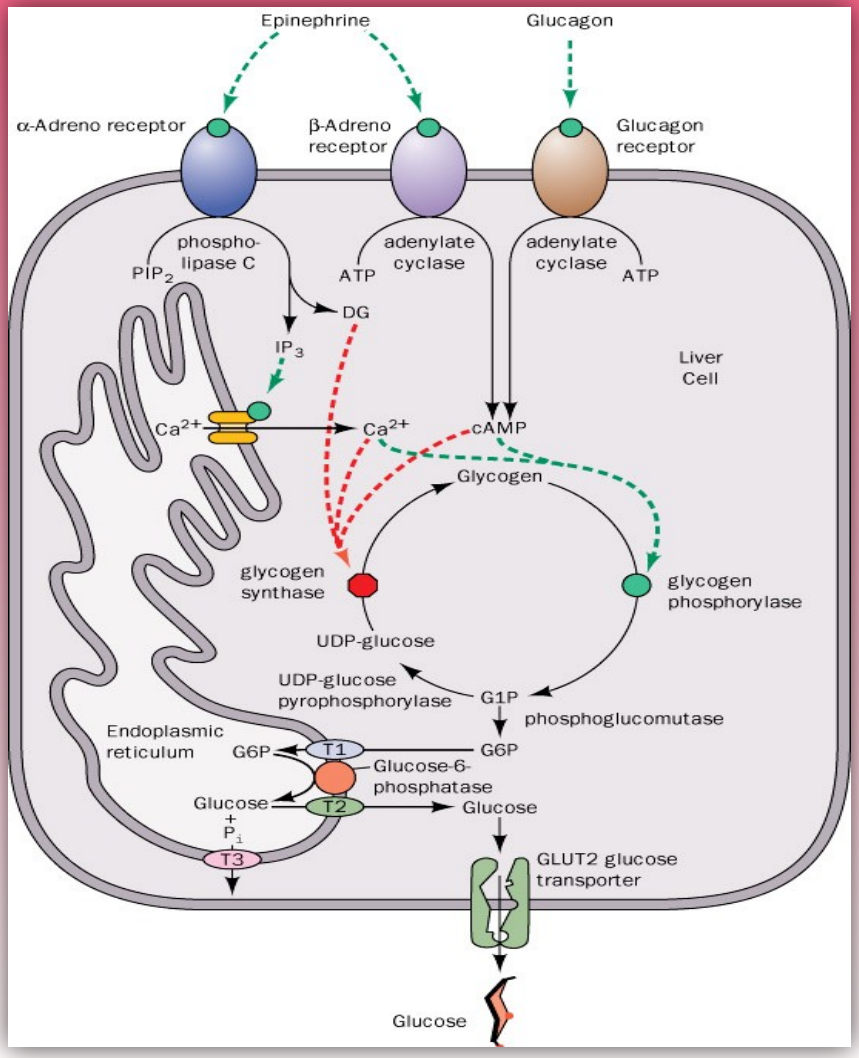
Comparison of the relative enzymatic activities of hexokinase and glucokinase over the physiological blood glucose range



The liver's response to stress: Stimulation of α-adrenoreceptors by epinephrine activates phospholipase C to hydrolyze PIP_2 to IP_3 and DAG

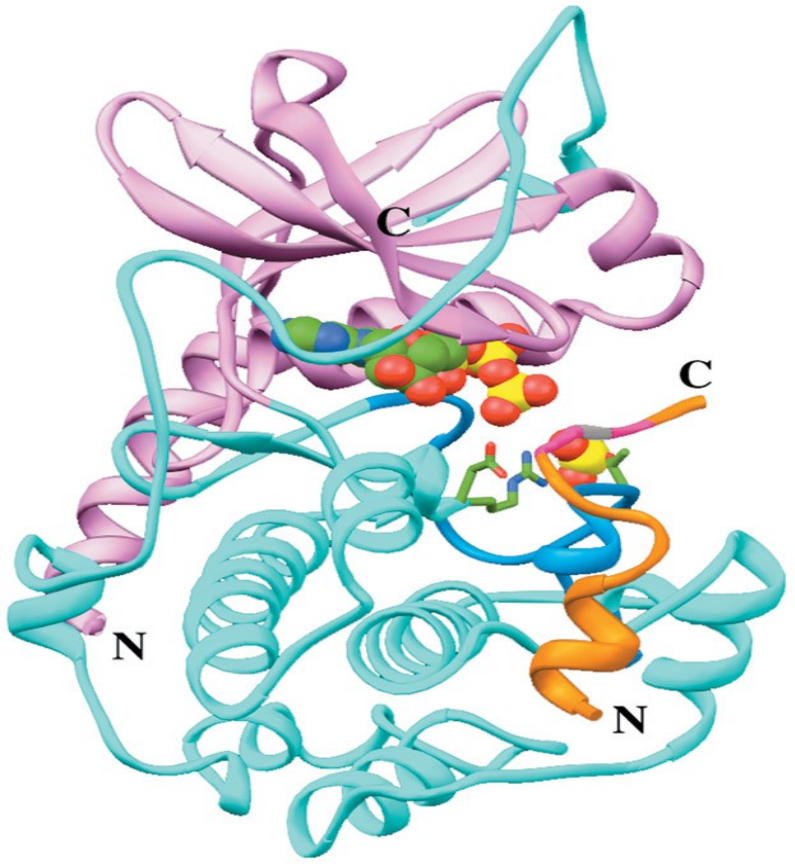


The liver's response to stress: The participation of two second messenger systems

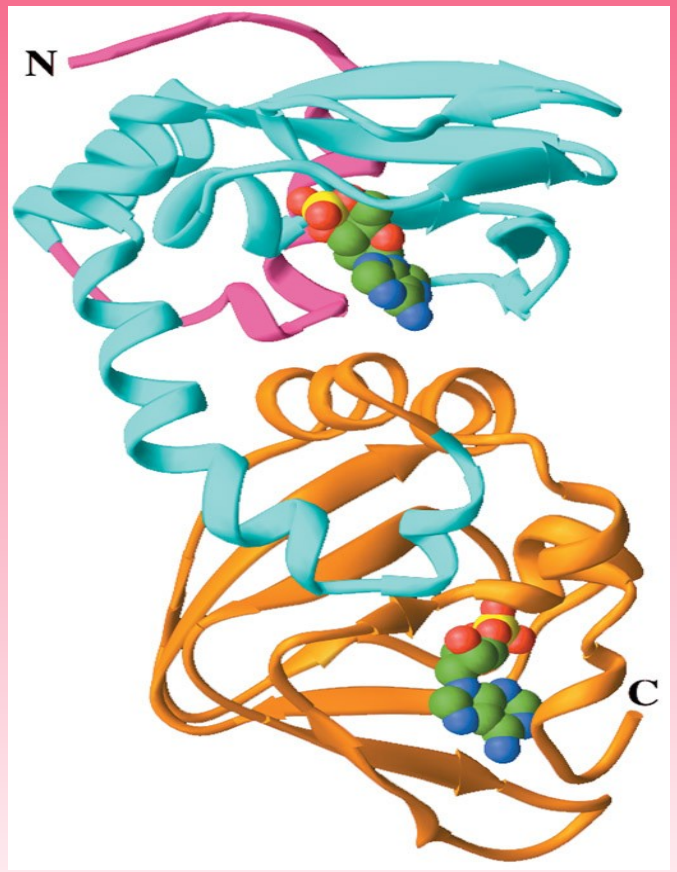


END

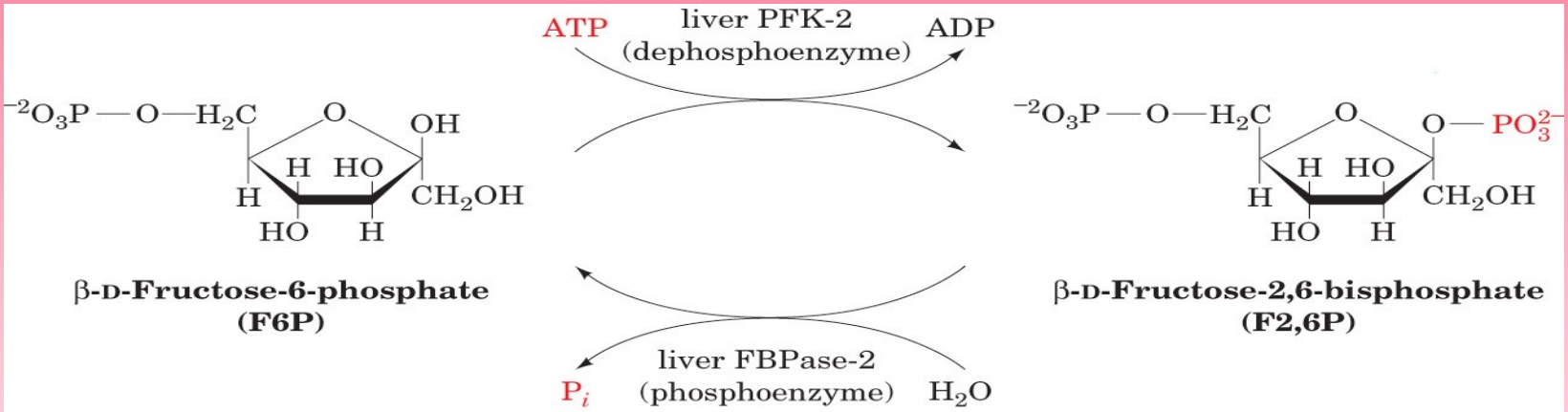
X-ray structure of the catalytic (C) subunit of mouse protein kinase A (PKA).



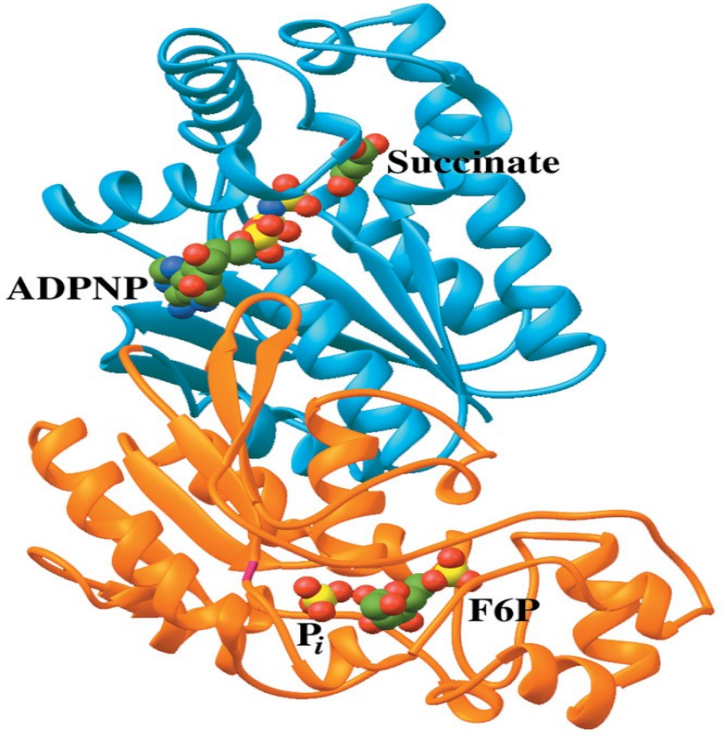
X-ray structure of the regulatory (R) subunit of bovine protein kinase A (PKA).



Formation and degradation of β-D-fructose-2,6-bisphosphate as catalyzed by PFK-2 and FBPase-2.



X-ray structure of the H256A mutant of rat testis PFK-2/FBPase-2.



The ADP concentration in human forearm muscles during rest and following exertion in normal individuals and those with McArdle's disease.