Biochemistry

Metabolism

22.11.2018 – 11.12.2018

Glycolysis

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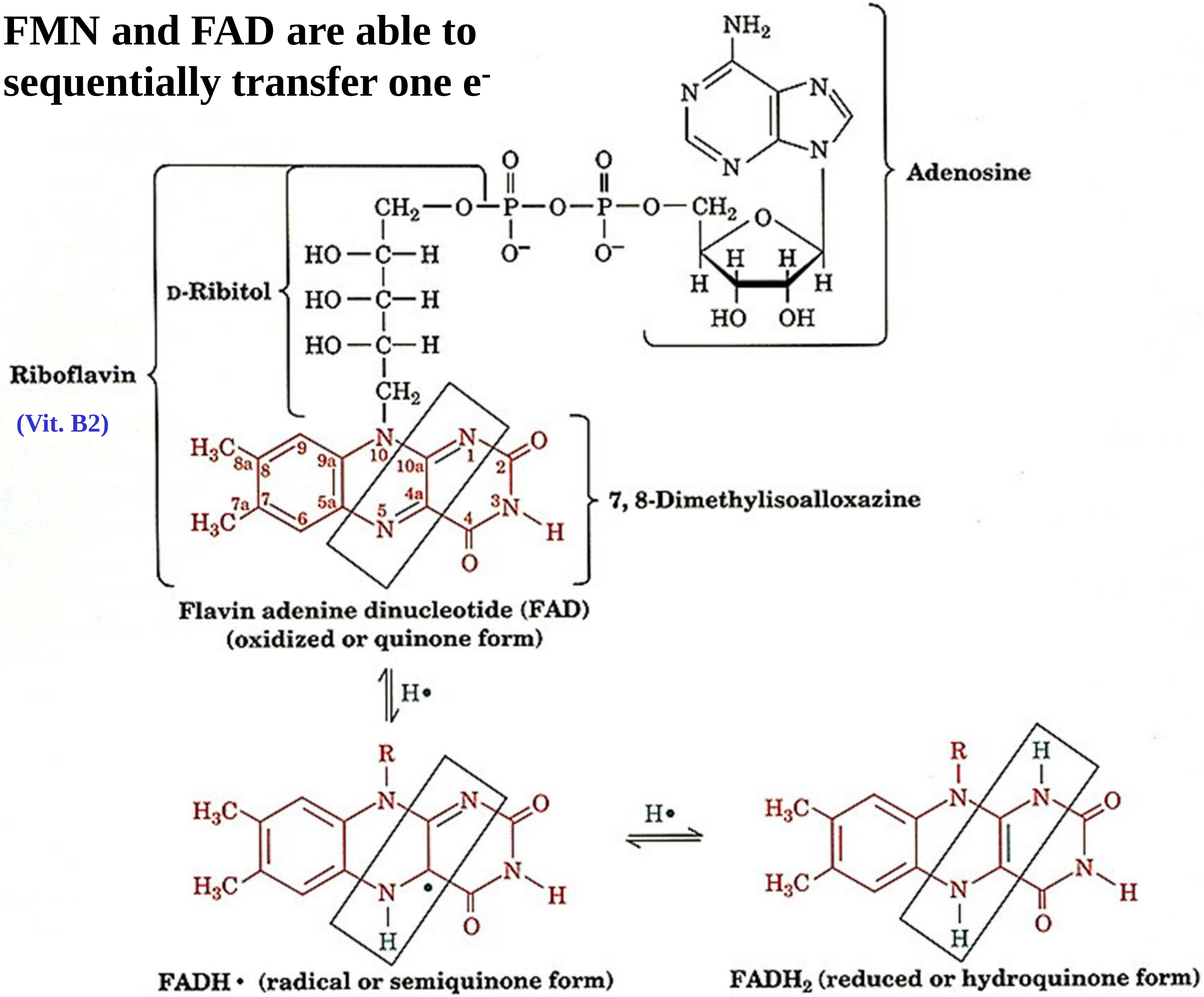
FMN and FAD are able to sequentially transfer one e$^-$



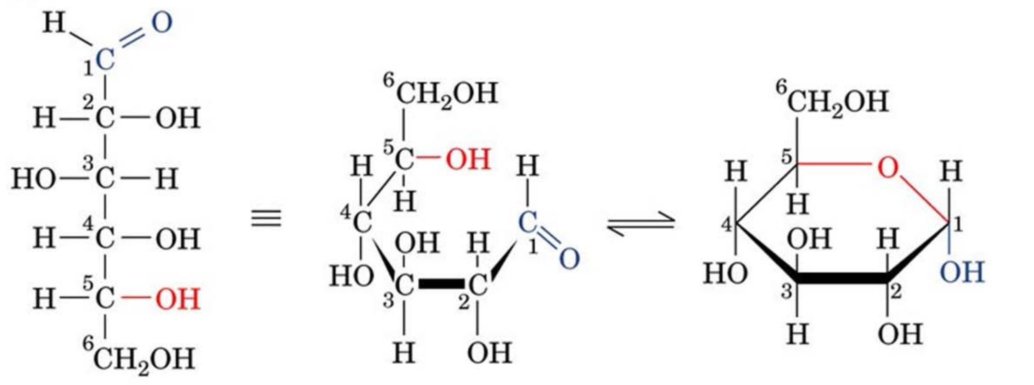
Biochemistry. Voet & Voet

TABLE 13–9 Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	*Flavin nucleotide*	*Text page(s)*
Acyl–CoA dehydrogenase	FAD	638
Dihydrolipoyl dehydrogenase	FAD	605
Succinate dehydrogenase	FAD	612
Glycerol 3-phosphate dehydrogenase	FAD	714–715
Thioredoxin reductase	FAD	869
NADH dehydrogenase (Complex I)	FMN	696–697
Glycolate oxidase	FMN	767

Lehninger Principles of Biochemistry. 5th Ed.
Nelson & Cox

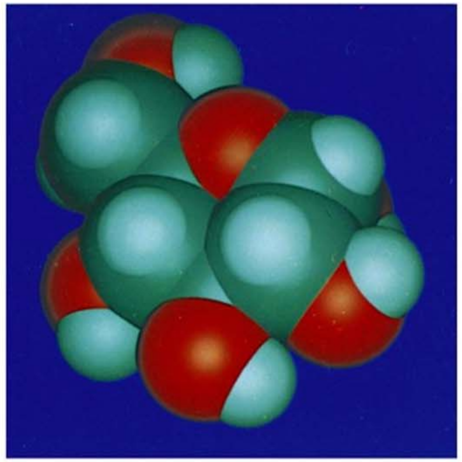
(a)



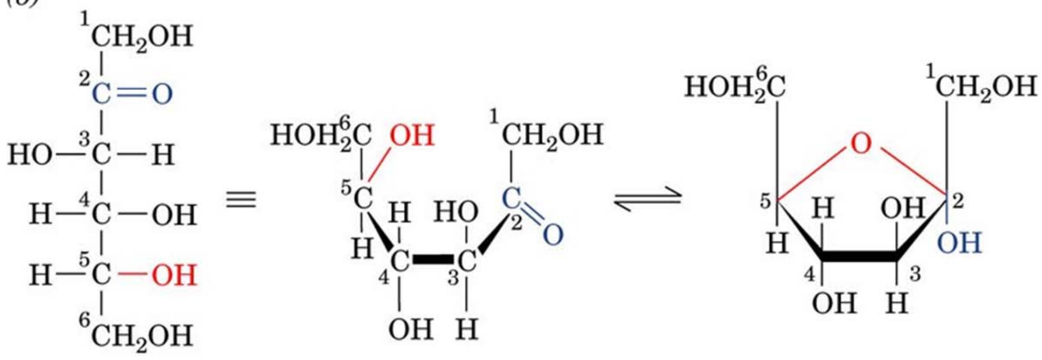
D-Glucose
(linear form)

α-D-Glucopyranose
(Haworth projection)

hemiacetal



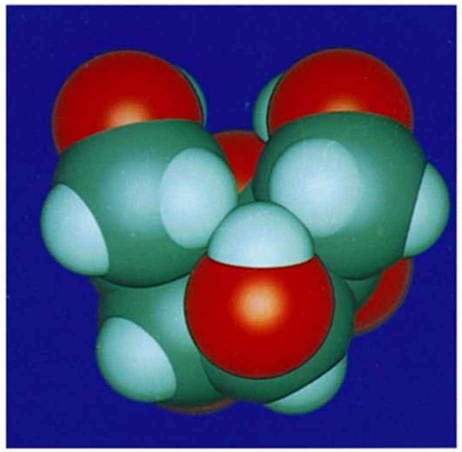
(b)



D-Fructose
(linear form)

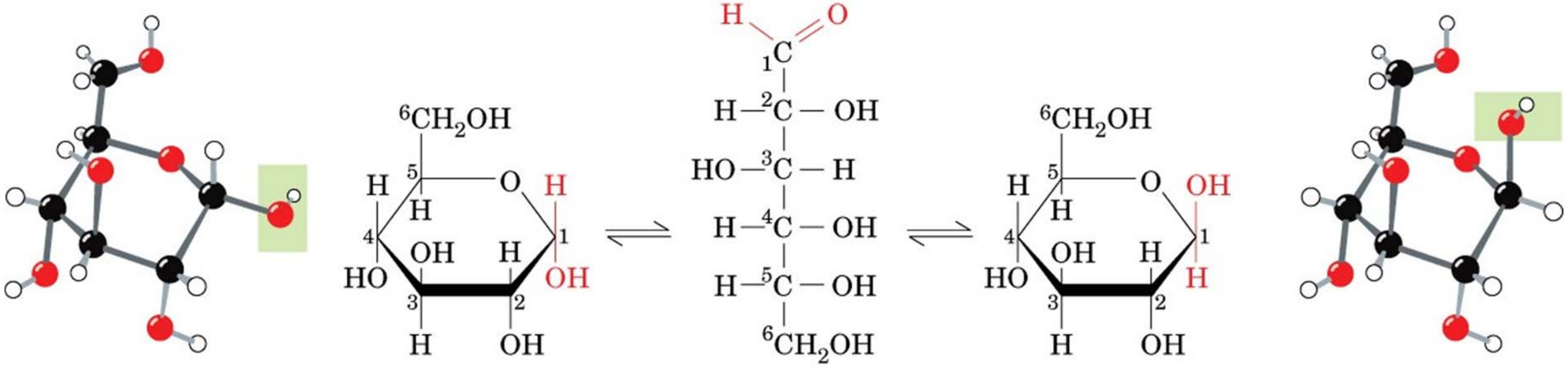
α-D-Fructofuranose
(Haworth projection)

hemiketal



Space-filling models courtesy of Robert Stodola, Fox Chase Cancer Center

Figure 11-4 Cyclization reactions for hexoses.



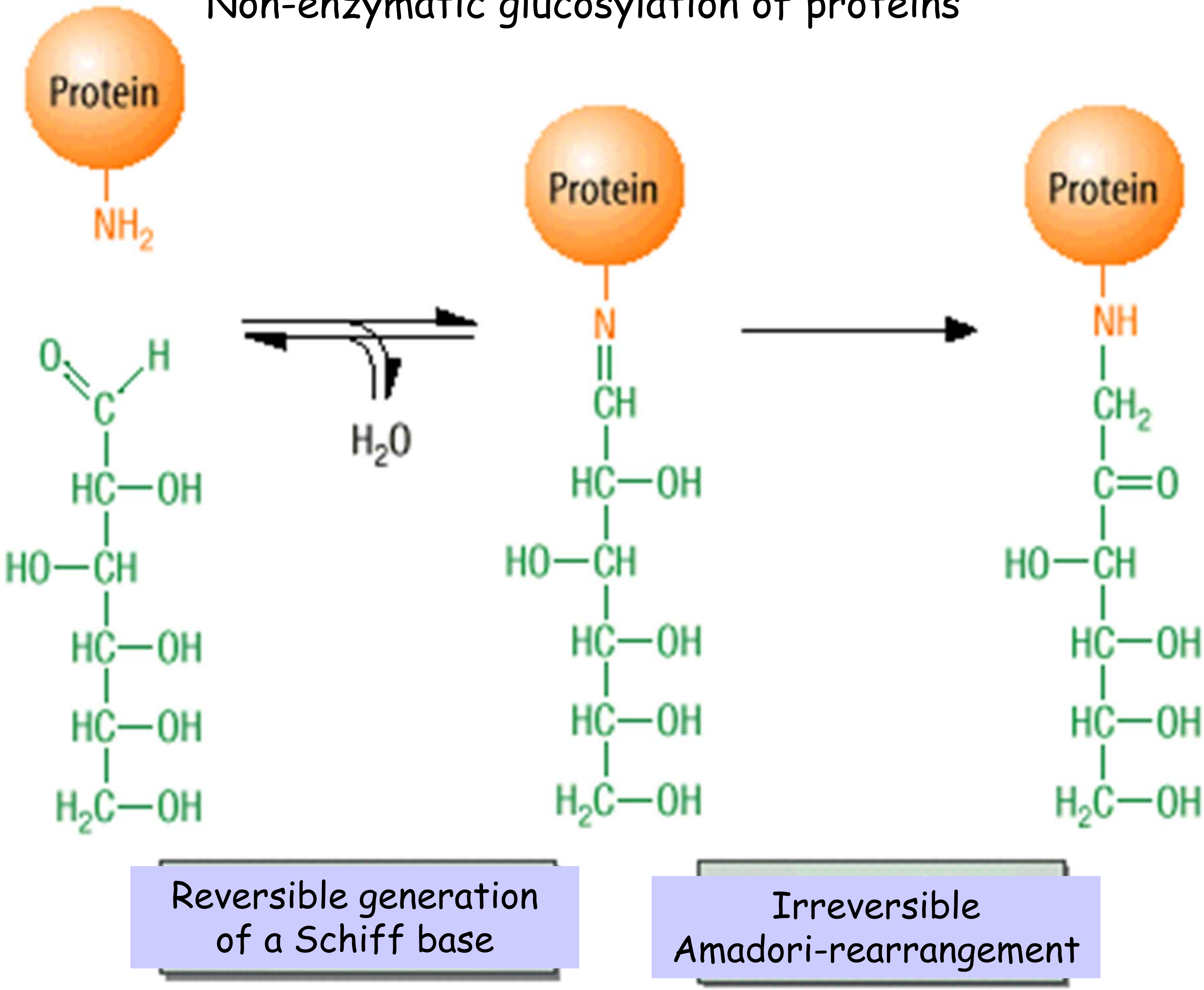
α-D-Glucopyranose

D-Glucose
(linear form)

β-D-Glucopyranose

Figure 11-5 The anomeric monosaccharides α-D-glucopyranose and β-D-glucopyranose, drawn as both Haworth projections and ball-and-stick models.

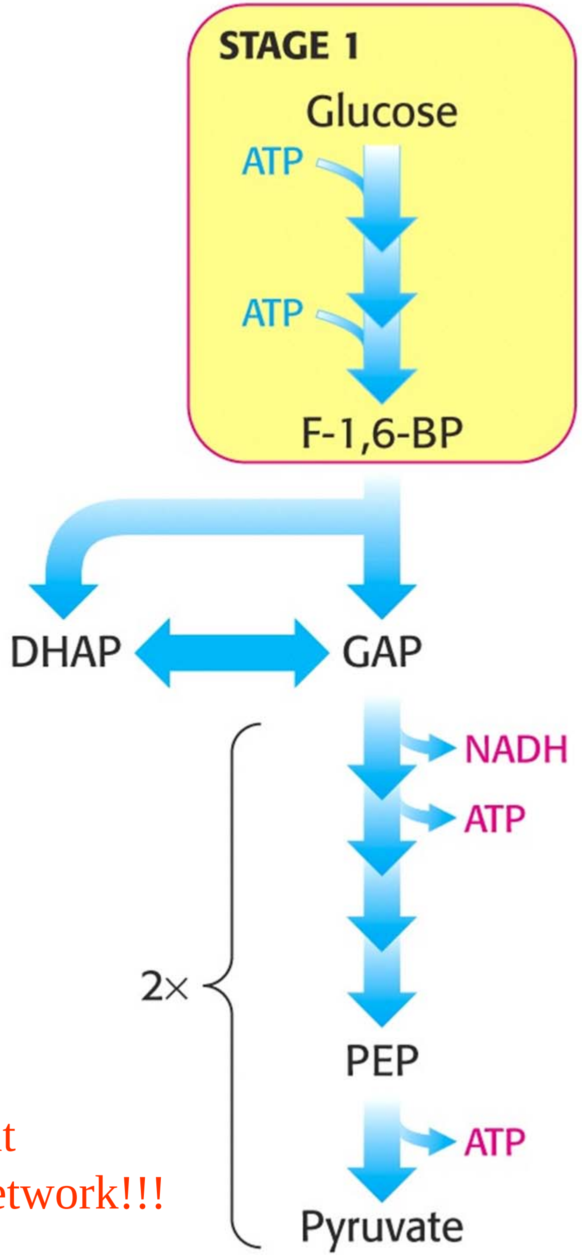
Non-enzymatic glucosylation of proteins

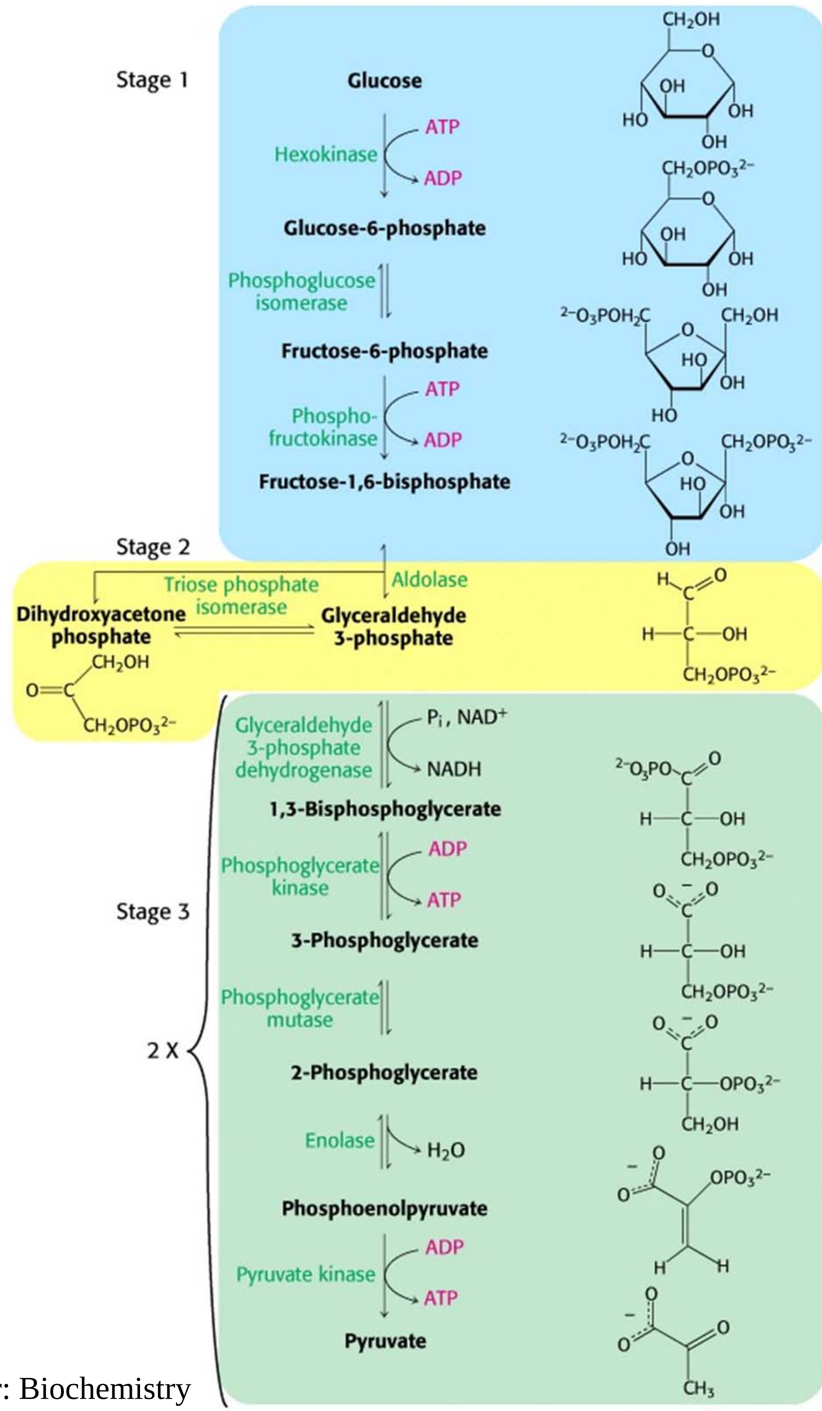


Glycolysis - overview

Preparatory phase:

Payoff phase:





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Not an one-way road but
a branched metabolic network!!!

Energetics of glycolysis

TABLE 16.3 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kcal mol^{-1} (kJ mol^{-1})	ΔG in kcal mol^{-1} (kJ mol^{-1})
1	Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H^+	Hexokinase	Phosphoryl transfer	−4.0 (−16.7)	−8.0 (−33.5)
2	Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+0.4 (+1.7)	−0.6 (−2.5)
3	Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−3.4 (−14.2)	−5.3 (−22.2)
4	Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetonephosphate + glyceraldehyde 3-phosphate	Aldolase	Aldol cleavage	+5.7 (+23.8)	−0.3 (−1.3)
5	Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization	+1.8 (+7.5)	+0.6 (+2.5)
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+1.5 (+6.3)	+0.6 (+2.5)
7	1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−4.5 (−18.8)	+0.3 (+1.3)
8	3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+1.1 (+4.6)	+0.2 (+0.8)
9	2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	Enolase	Dehydration	+0.4 (+1.7)	−0.8 (−3.3)
10	Phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−7.5 (−31.4)	−4.0 (−16.7)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

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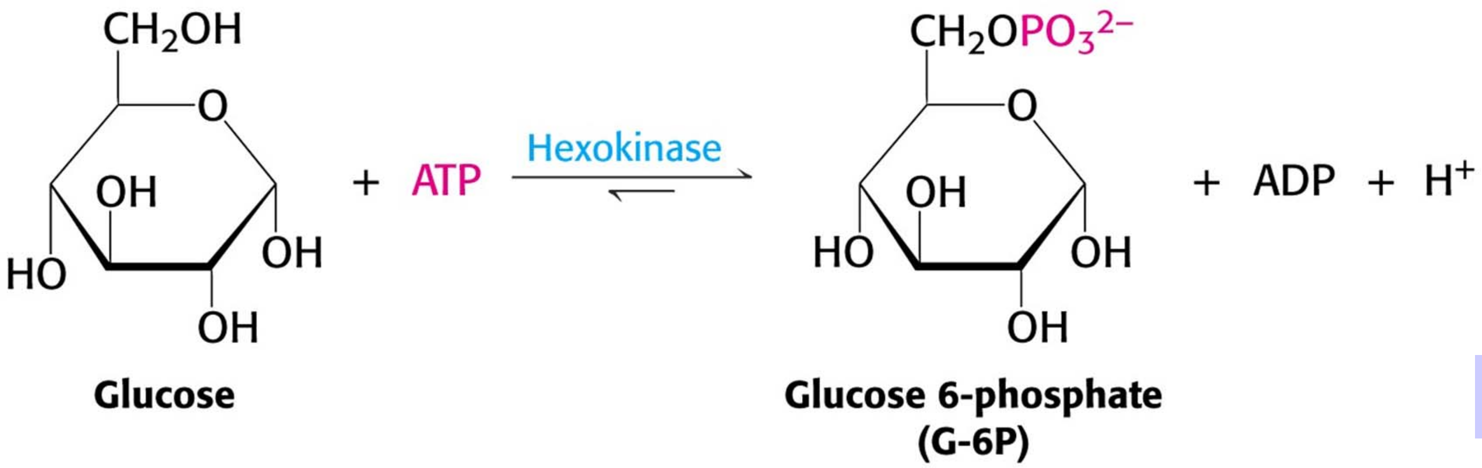
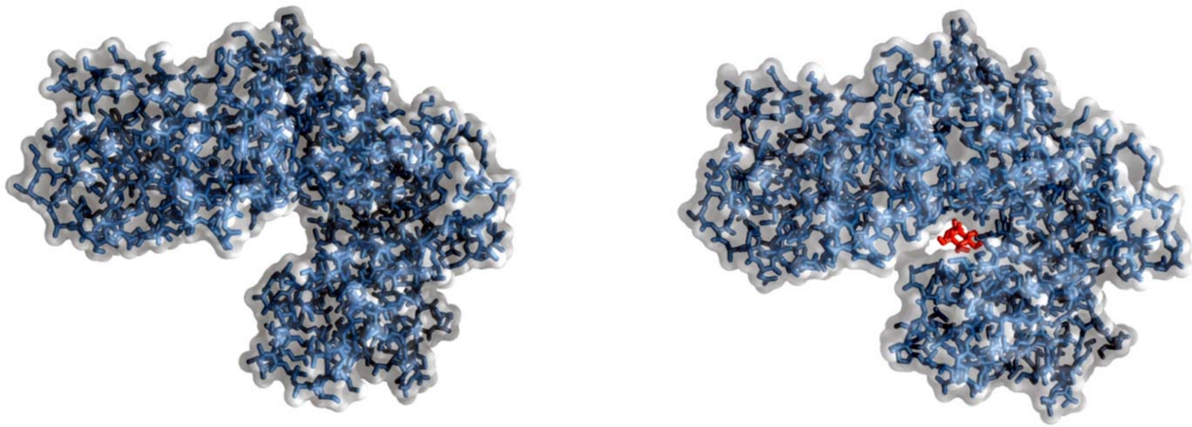
Glycolytic steps: Phosphorylation catalysed by **hexokinase**



„Isoenzyme"

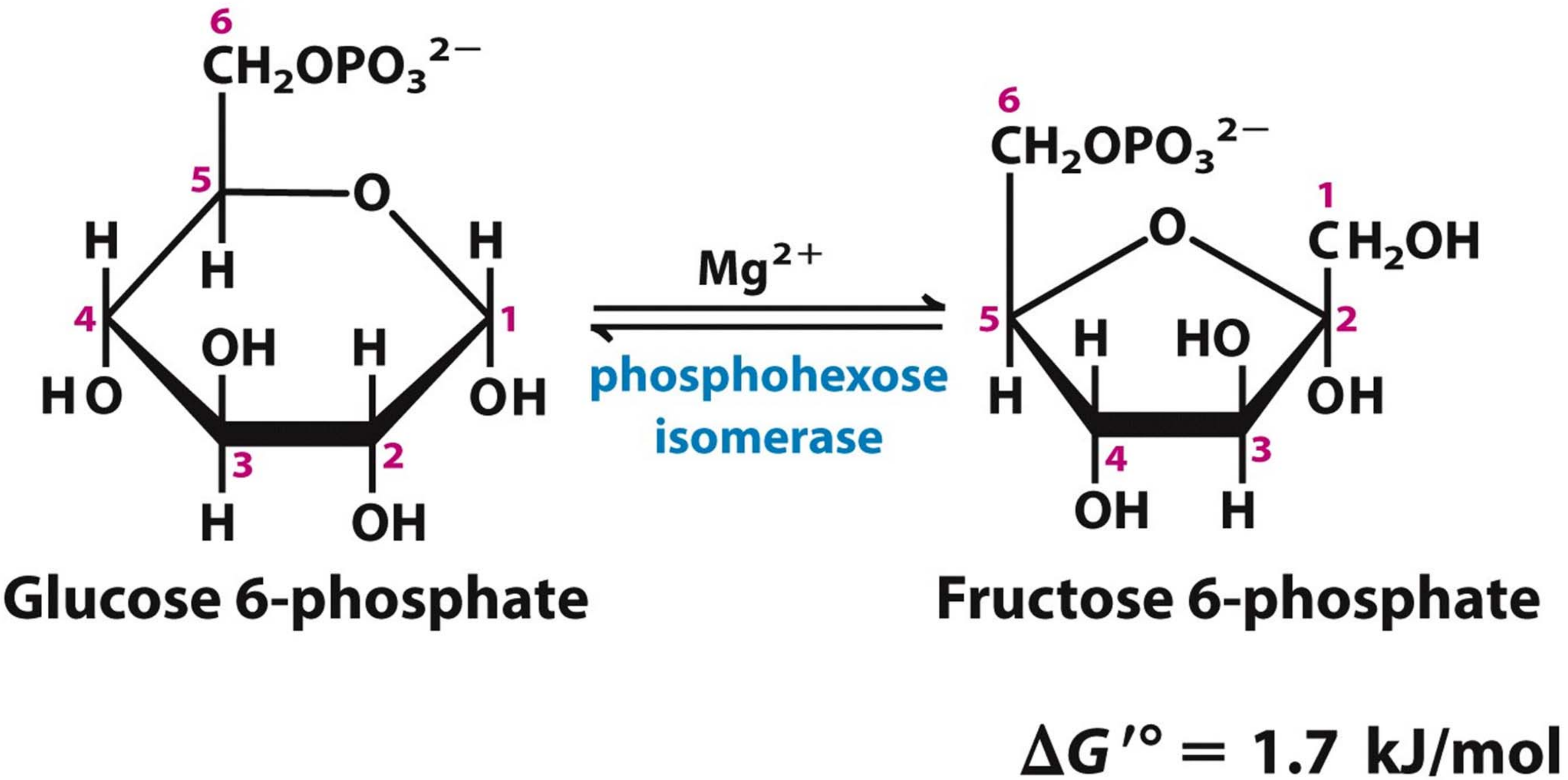
- ubiquitous
- unspecific (catalyses phosphorylation of several hexoses)
- co-substrate Mg-ATP-complex
- $K_{MGlc} < 100\ \mu M$!!!

•Note:
in the liver **Glucokinase**:
$K_{MGlc} = 10\ mM$



Induced fit Berg, Tymoczko, Stryer: Biochemistry

Glycolytic steps: Isomerisation catalysed by **glucose phosphate isomerase**

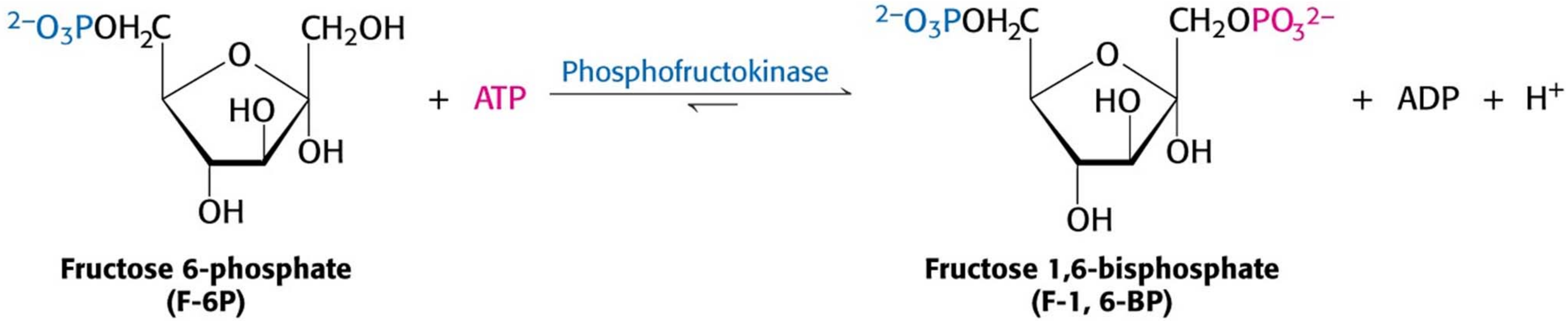


Unnumbered 14 p532b
Lehninger Principles of Biochemistry, Fifth Edition

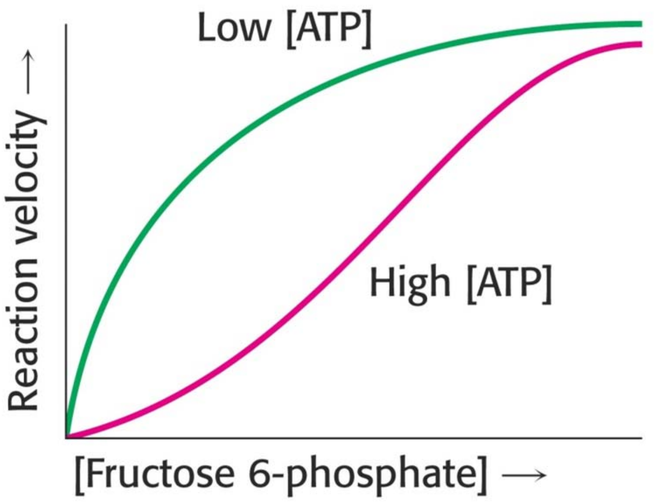
Reaction mechanism:

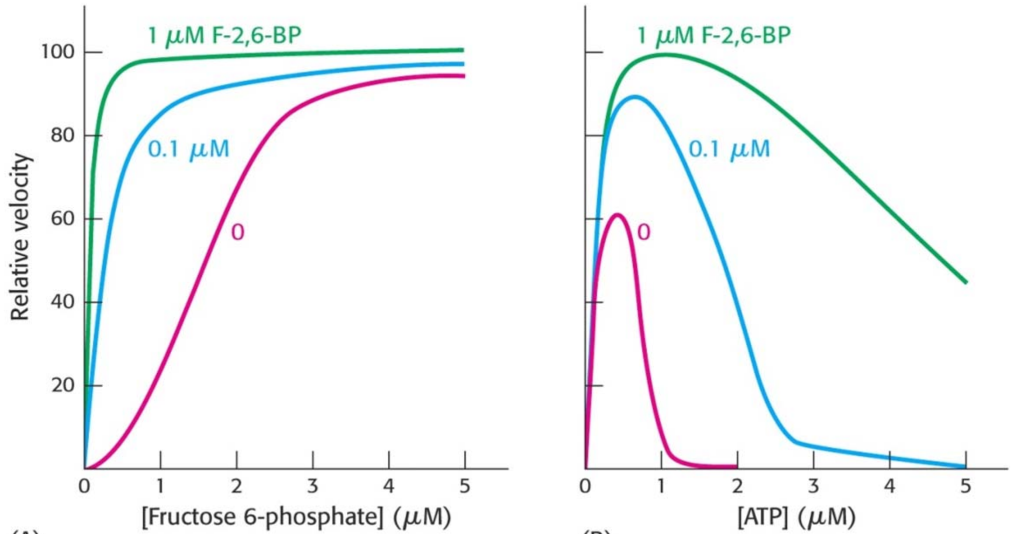
General acid/base catalysis

Glycolitic steps: Phosphorylation catalysed by **phosphofructokinase (PFK)**
(rate-limiting step)



Allosteric modulators of PFK: ATP, AMP, Citrate, H^+, F-2,6-BP (Activators, Inhibitors)

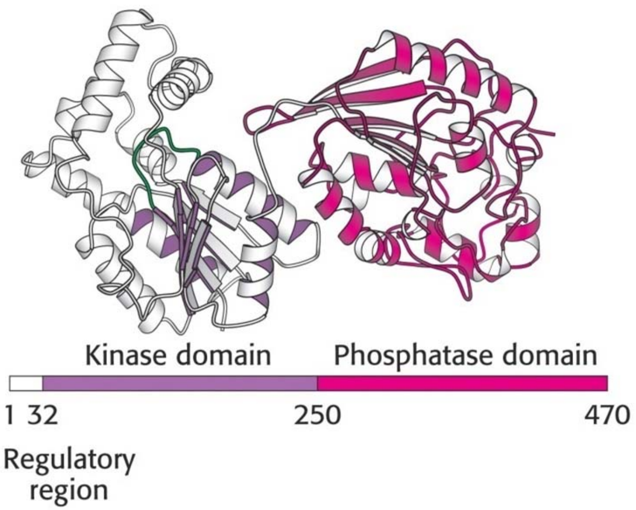


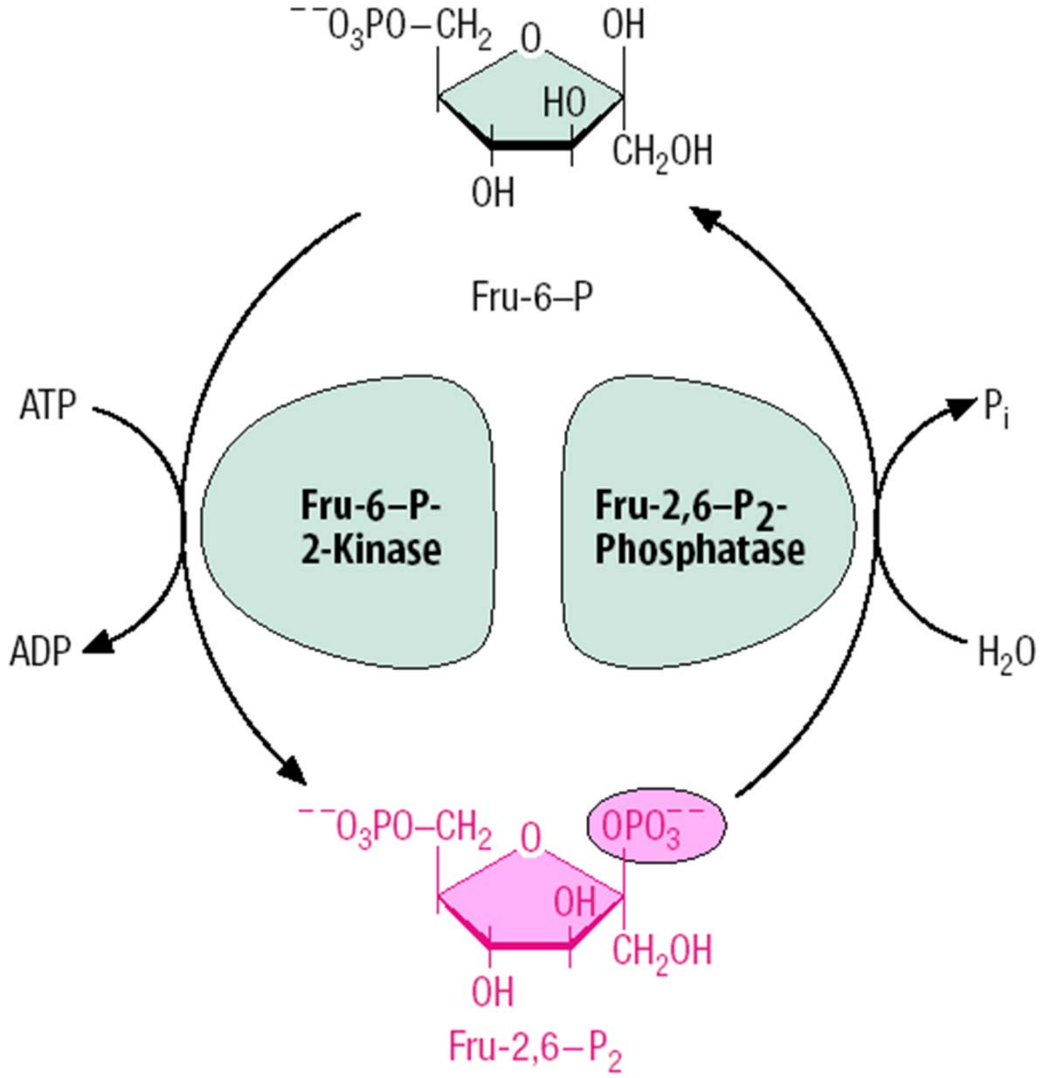


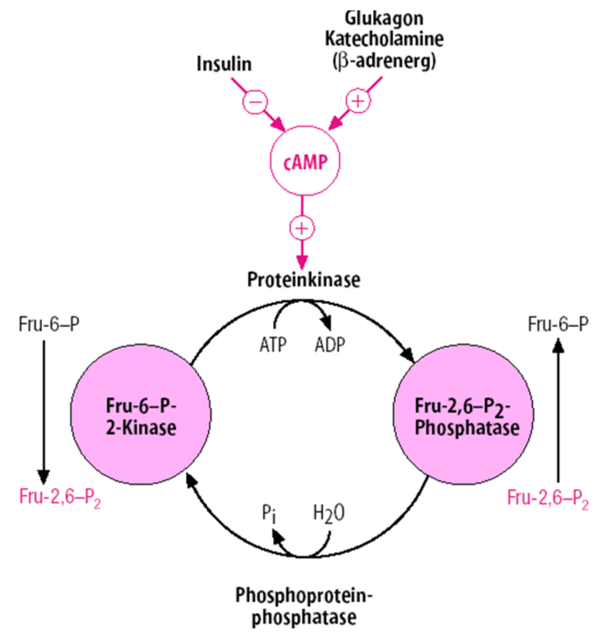
(A) (B)

Regulation of F1,6BPase is mirror-inverted but opposite !!!

PFK2: a bifunctional (tandem)-enzyme

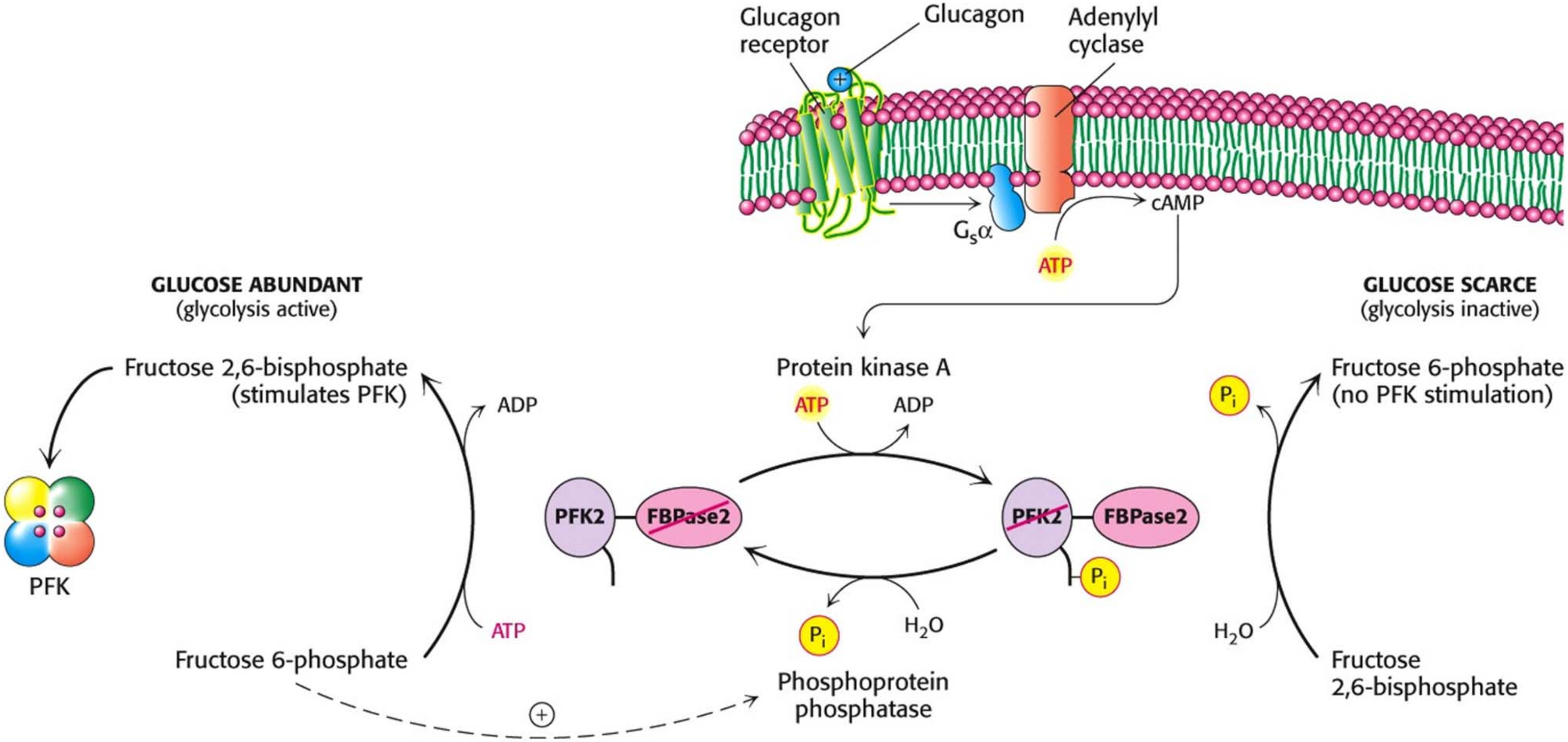






Hormone regulation of the enzyme is tissue-specific!!!

cAMP-mediated hormonal regulation of PFK via F2,6BP



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General mechanism of the activation of effector proteins associated with GPCRs

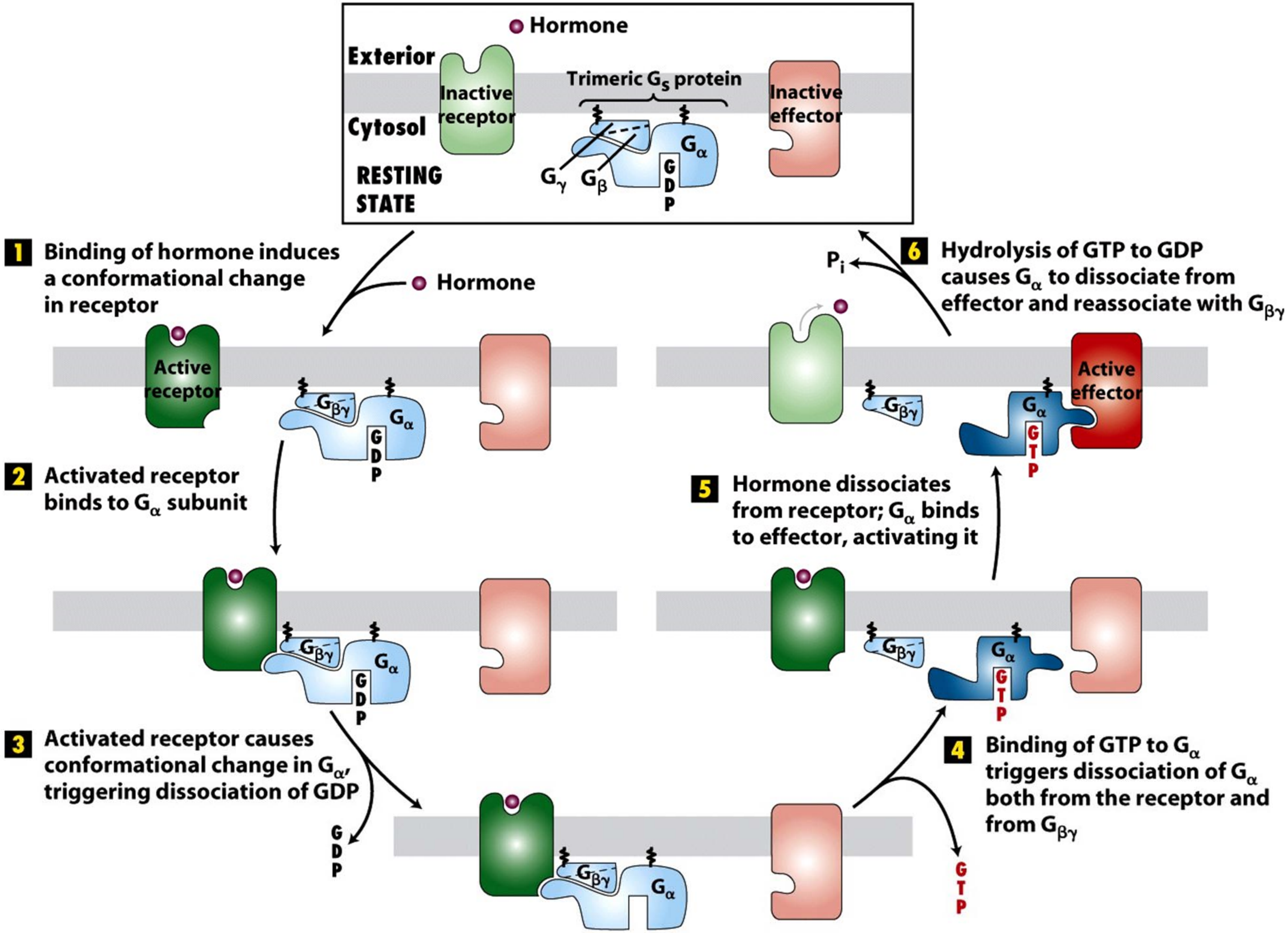


Figure 15-13
Molecular Cell Biology, Sixth Edition

Hormone-induced activation and inhibition of adenylyl cyclase in adipocytes

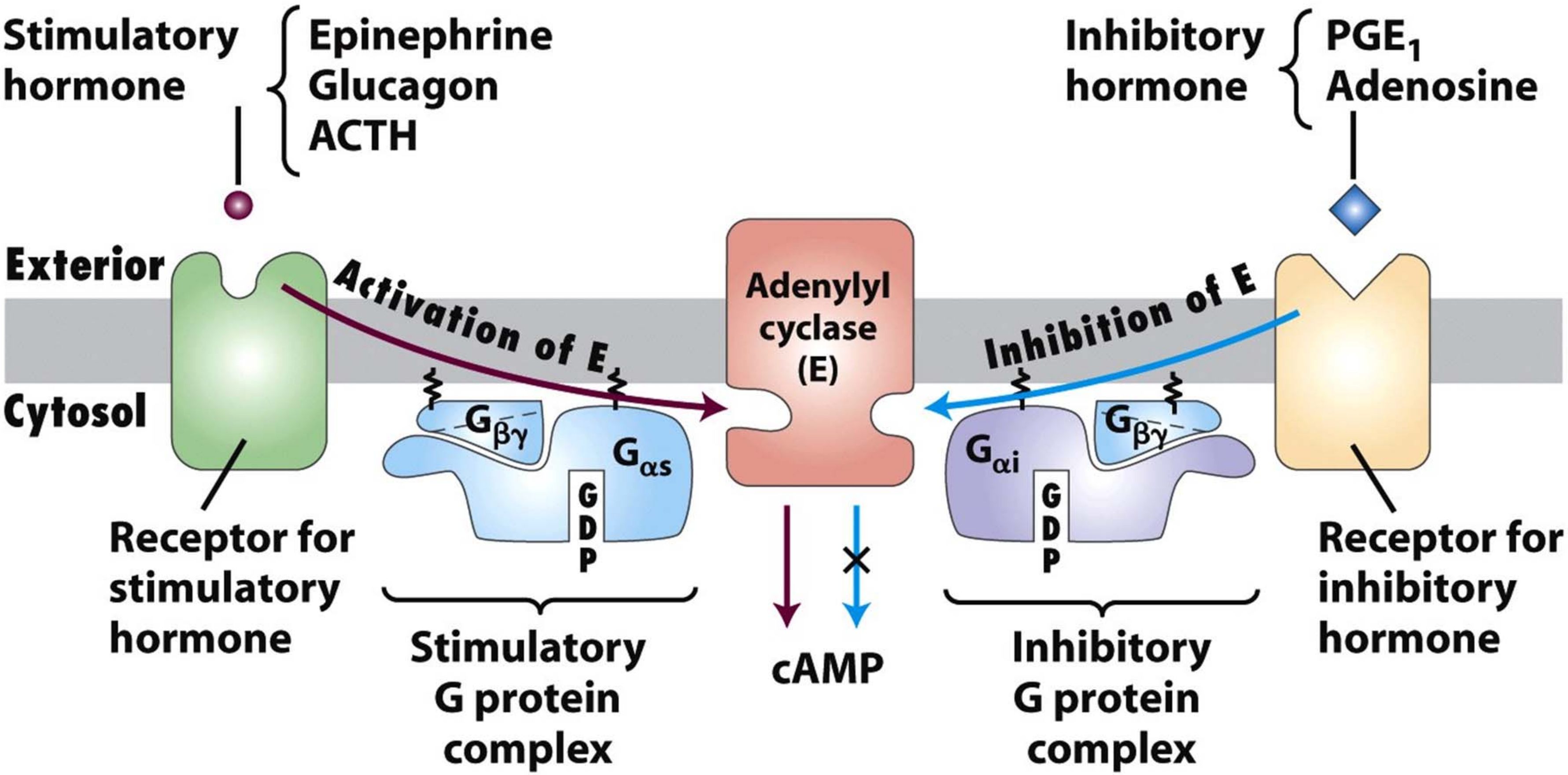


Figure 15-21
Molecular Cell Biology, Sixth Edition

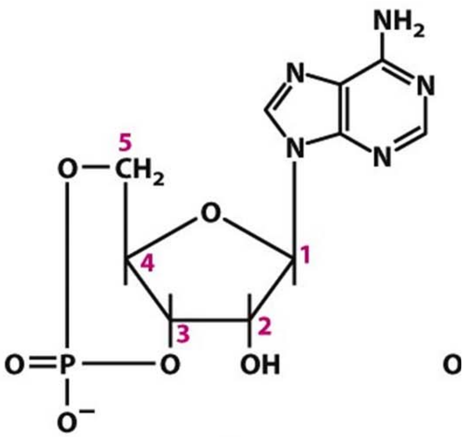
Lodish et al. Molecular Biology of the Cell

TABLE 15.2 G-protein families and their functions

G_α class	Initiating signal	Downstream signal
$G_{\alpha s}$	β-Adrenergic amines, glucagon, parathyroid hormone, many others	Stimulates adenylate cyclase
$G_{\alpha i}$	Acetylcholine, α-adrenergic amines, many neurotransmitters	Inhibits adenylate cyclase
$G_{\alpha t}$	Photons	Stimulates cGMP phosphodiesterase
$G_{\alpha q}$	Acetylcholine, α-adrenergic amines, many neurotransmitters	Increases IP_3 and intracellular calcium PLC-β
$G_{\alpha 13}$	Thrombin, other agonists	Stimulates Na^+ and H^+ exchange

Source: Z. Farfel, H. R. Bourne, and T. Iiri. *N. Engl. J. Med.* 340(1999):1012.

Adenylyl cyclase generates the second messenger cAMP using ATP as a substrate



3′,5′-Cyclic AMP (cAMP)

Activates protein kinase A (PKA)

Figure 15-9
Molecular Cell Biology, Sixth Edition

Structure and activation of Protein kinase A by cAMP

Protein kinase A (PKA)

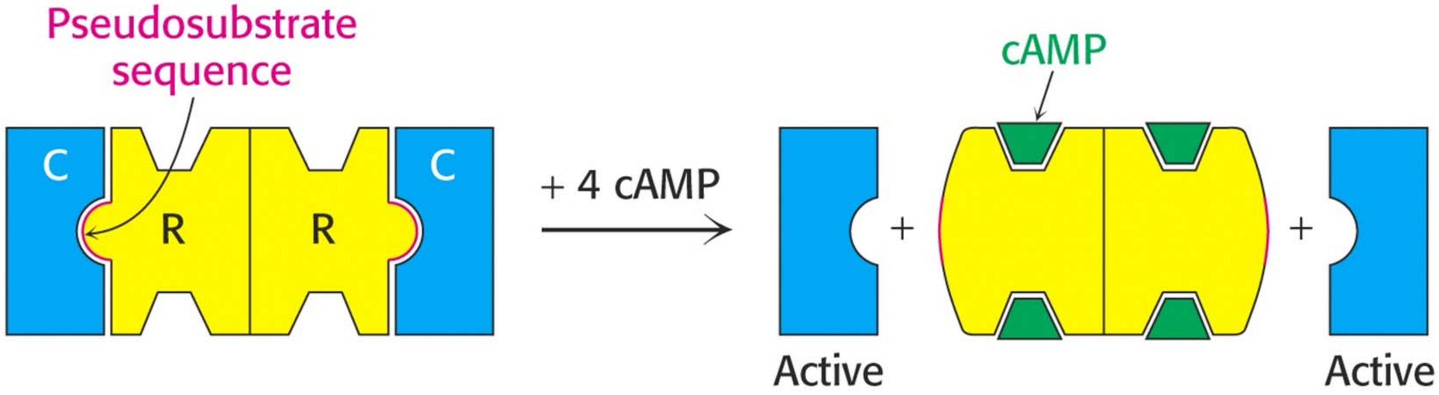


TABLE 15-2 Cellular Responses to Hormone-Induced Rise in cAMP in Various Tissues*

TISSUE	HORMONE INDUCING RISE IN cAMP	CELLULAR RESPONSE
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of glycogen synthesis; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle	Epinephrine	Increase in contraction rate
Thyroid gland	TSH	Secretion of thyroxine
Bone	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

*Nearly all the effects of cAMP are mediated through protein kinase A (PKA), which is activated by binding of cAMP.
SOURCE: E. W. Sutherland, 1972, *Science* **177**:401.

Table 15-2
Molecular Cell Biology, Sixth Edition

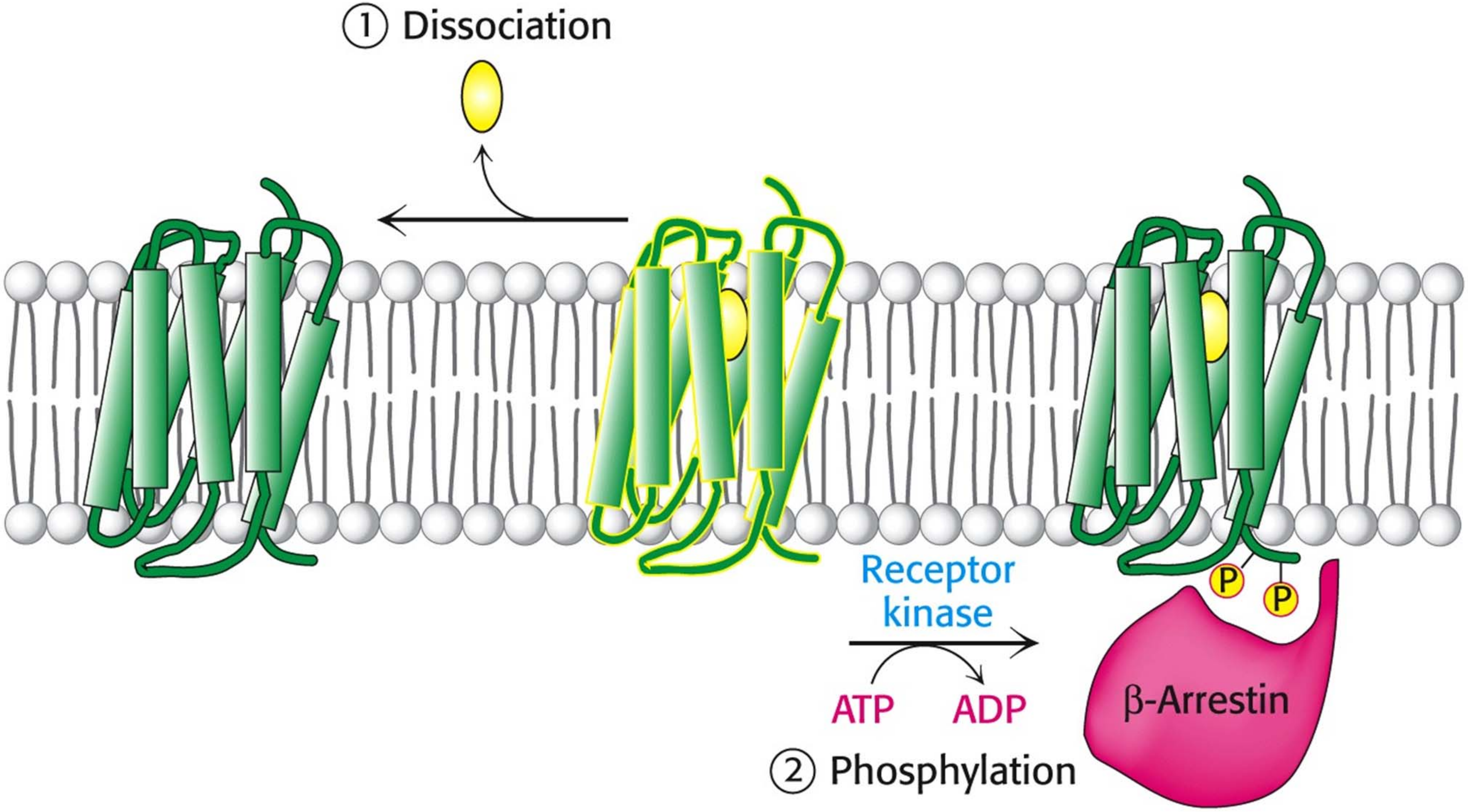
TABLE 12–3 Some Enzymes and Other Proteins Regulated by cAMP-Dependent Phosphorylation (by PKA)

Enzyme/protein	*Sequence phosphorylated**	*Pathway/process regulated*
Glycogen synthase	RASCTSSS	Glycogen synthesis
Phosphorylase *b* kinase		
α subunit	VEFRRLSI	Glycogen breakdown
β subunit	RTKRSGSV	Glycogen breakdown
Pyruvate kinase (rat liver)	GVLRRASVAZL	Glycolysis
Pyruvate dehydrogenase complex (type L)	GYLRRASV	Pyruvate to acetyl-CoA
Hormone-sensitive lipase	PMRRSV	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRGSSIPQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FIGRRQSL	Synthesis of L-DOPA, dopamine, norepinephrine, and epinephrine
Histone H1	AKRKASGPPVS	DNA condensation
Histone H2B	KKAKASRKESYSVYVYK	DNA condensation
Cardiac phospholamban (cardiac pump regulator)	AIRRAST	Intracellular [Ca^{2+}]
Protein phosphatase-1 inhibitor-1	IRRRRPTP	Protein dephosphorylation
PKA consensus sequence†	XR(R/K)X(S/T)B	Many

*The phosphorylated S or T residue is shown in red. All residues are given as their one-letter abbreviations (see Table 3–1).
†X is any amino acid; B is any hydrophobic amino acid.

Berg, Tymoczko, Stryer: Biochemistry, 2002

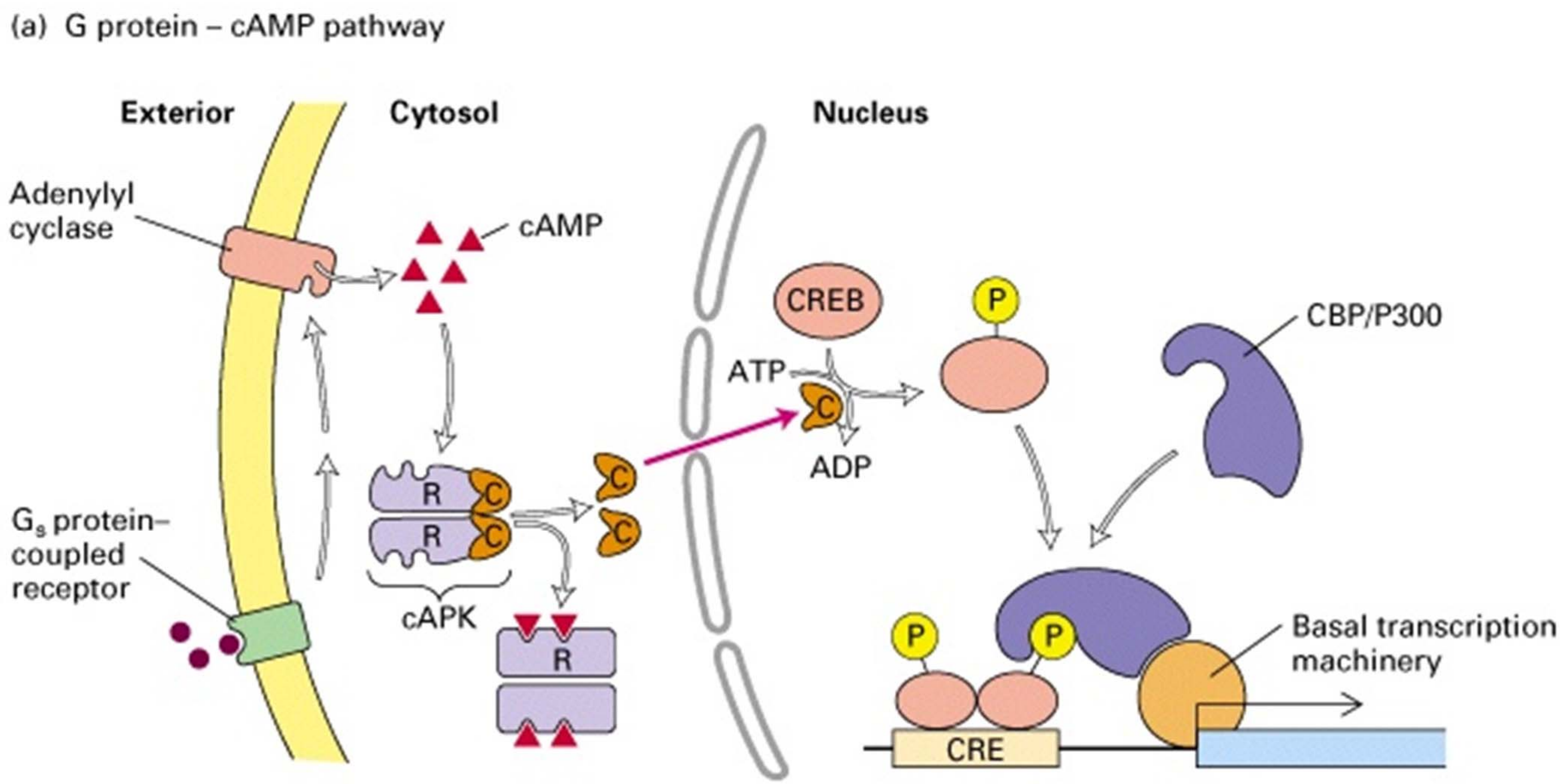
Termination/desensitization of the signal transduction process



3. Phosphodiesterase (PDE) catalyses hydrolysis of cAMP (calcium-dependent)
4. GTP-hydrolysis

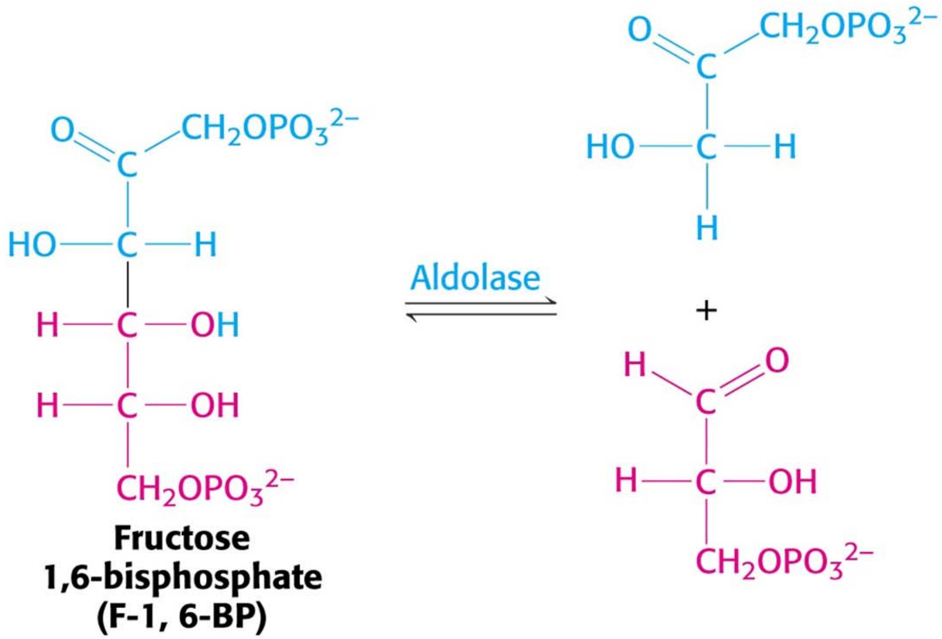
Berg, Tymoczko, Stryer: Biochemistry, 2002

CREB links cAMP signals to transcription



Lodish et al. Molecular Biology of the Cell

Glycolytic steps: Aldol cleavage catalysed by **aldolase**

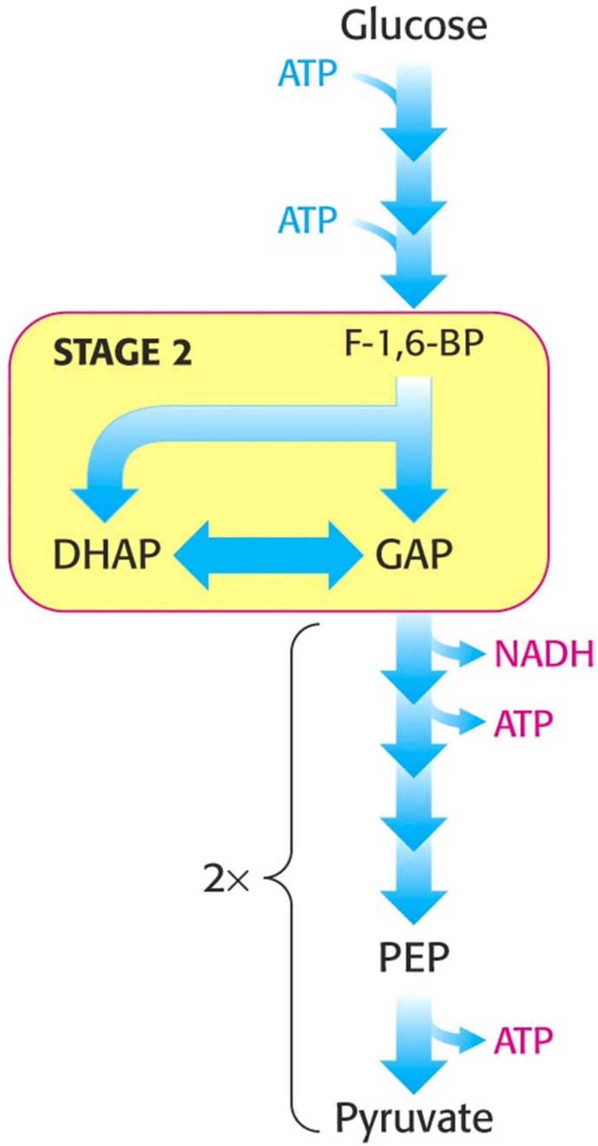


Dihydroxyaceton phosphate (DHAP)

96%

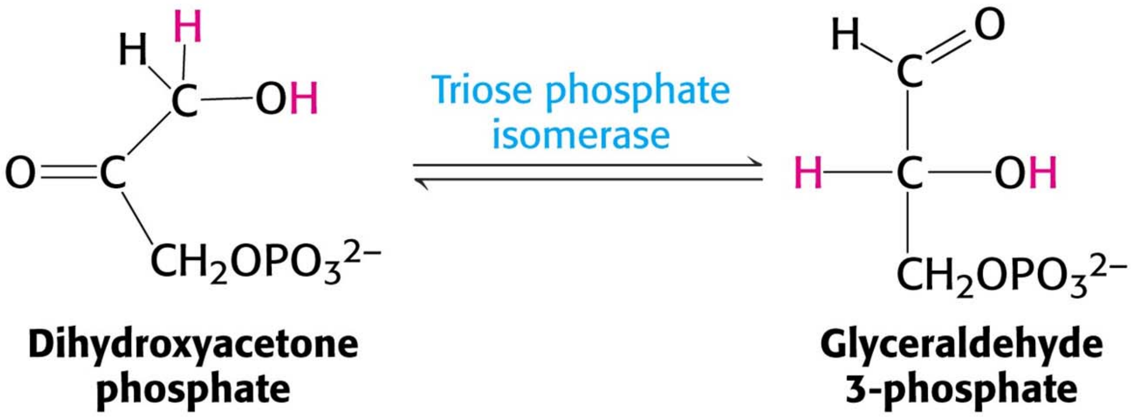
Glyceraldehyde 3-phosphate (GAP)

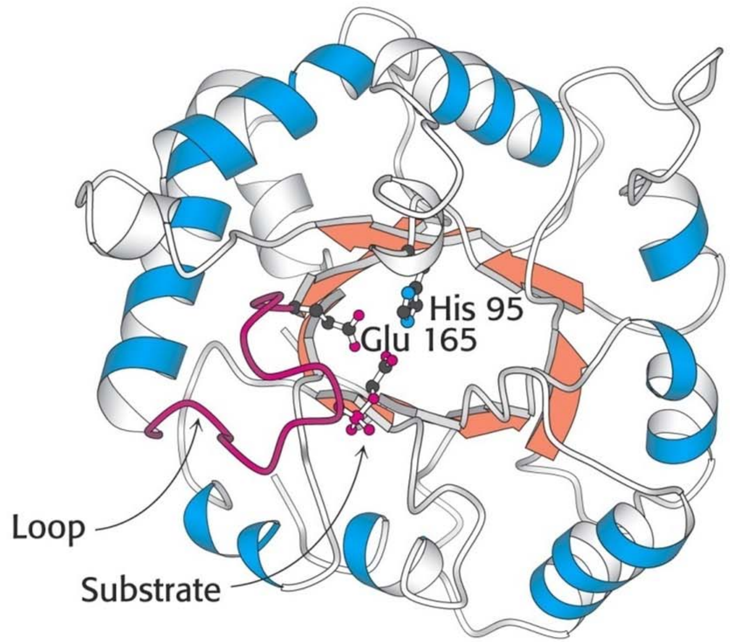
4%



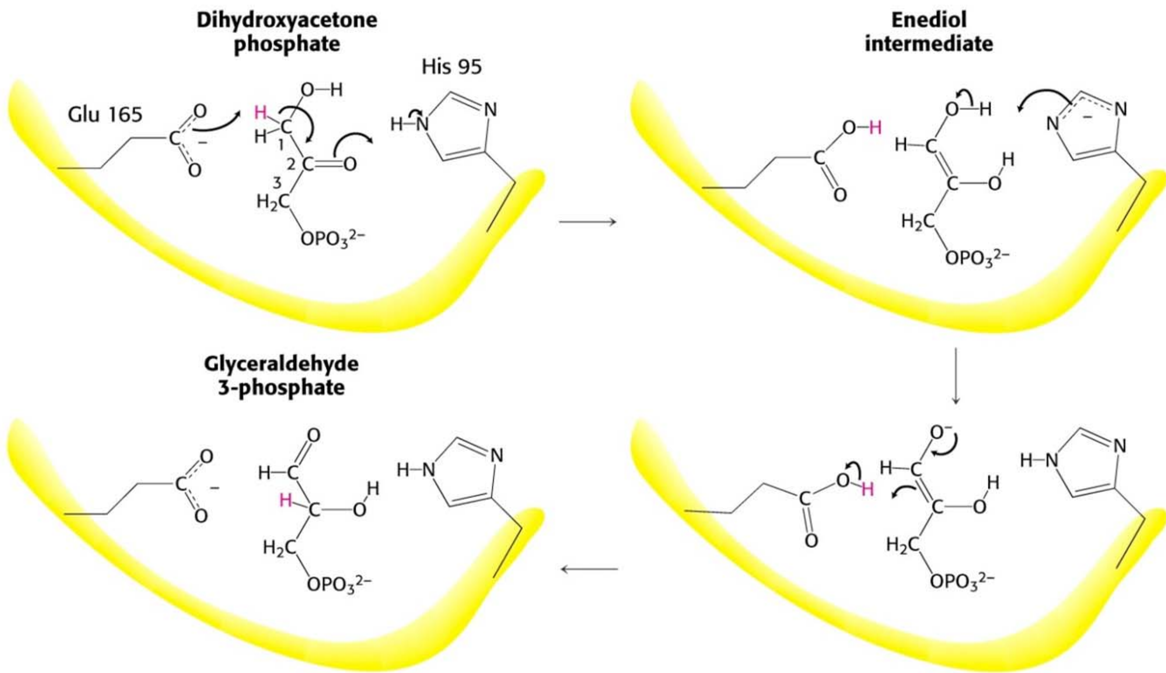
Berg, Tymoczko, Stryer: Biochemistry

Glyolytic steps: Isomerisation catalysed by **triose phosphate isomerase (TIM)**



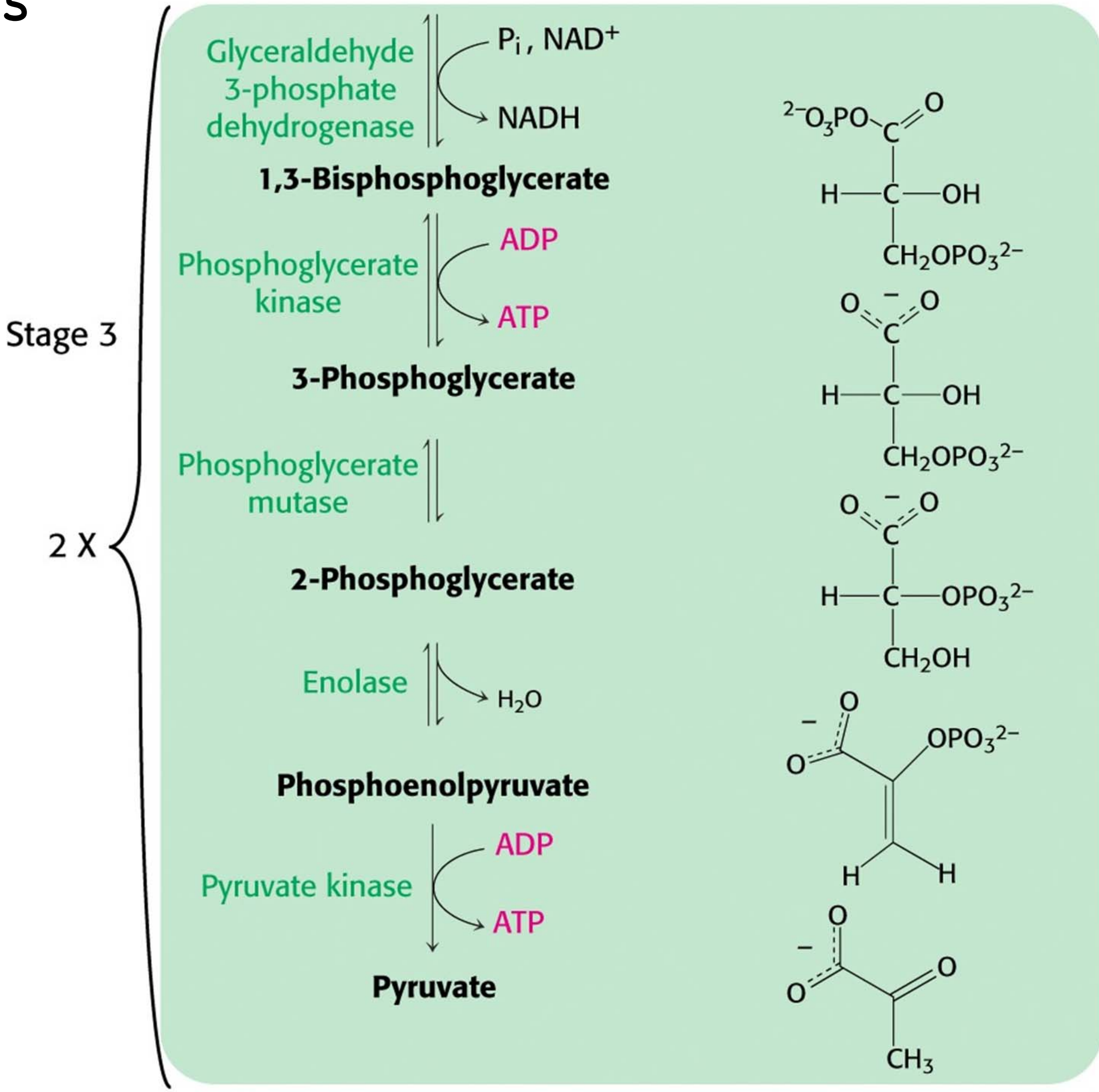


αβ-Barrel



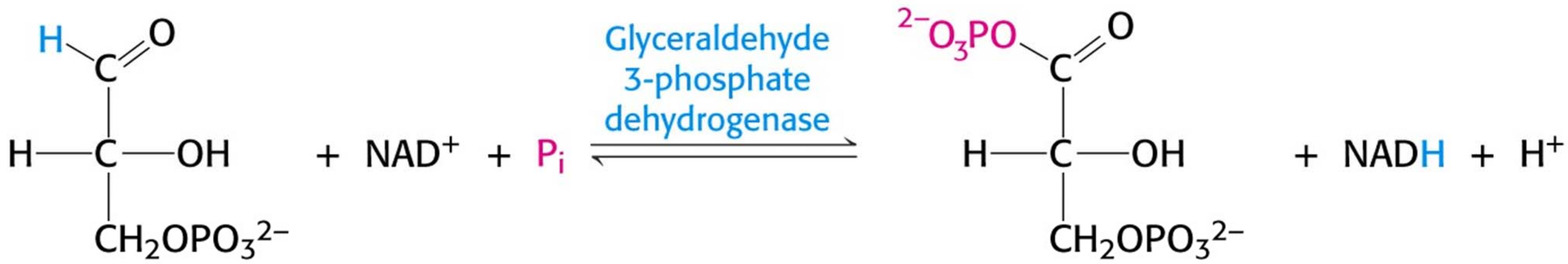
- catalysis via an endiol-intermediate
- Mech: general acid/base catalysis
- K=[GAP]/[DHAP]=$4{,}7 \times 10^{-2}$
  [DHAP]>> [GAP]
- Enzyme works diffusion controlled
- $k_{cat}/K_M = 2 \times 10^{8}\ M^{-1}s^{-1}$

Glycolysis



Stage 3
2 X
Glyceraldehyde 3-phosphate dehydrogenase
P_i, NAD^+
NADH
1,3-Bisphosphoglycerate
Phosphoglycerate kinase
ADP
ATP
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
H_2O
Phosphoenolpyruvate
Pyruvate kinase
ADP
ATP
Pyruvate

Glycolytic steps: Oxidative phosphorylation catalysed by **GAP-DH**



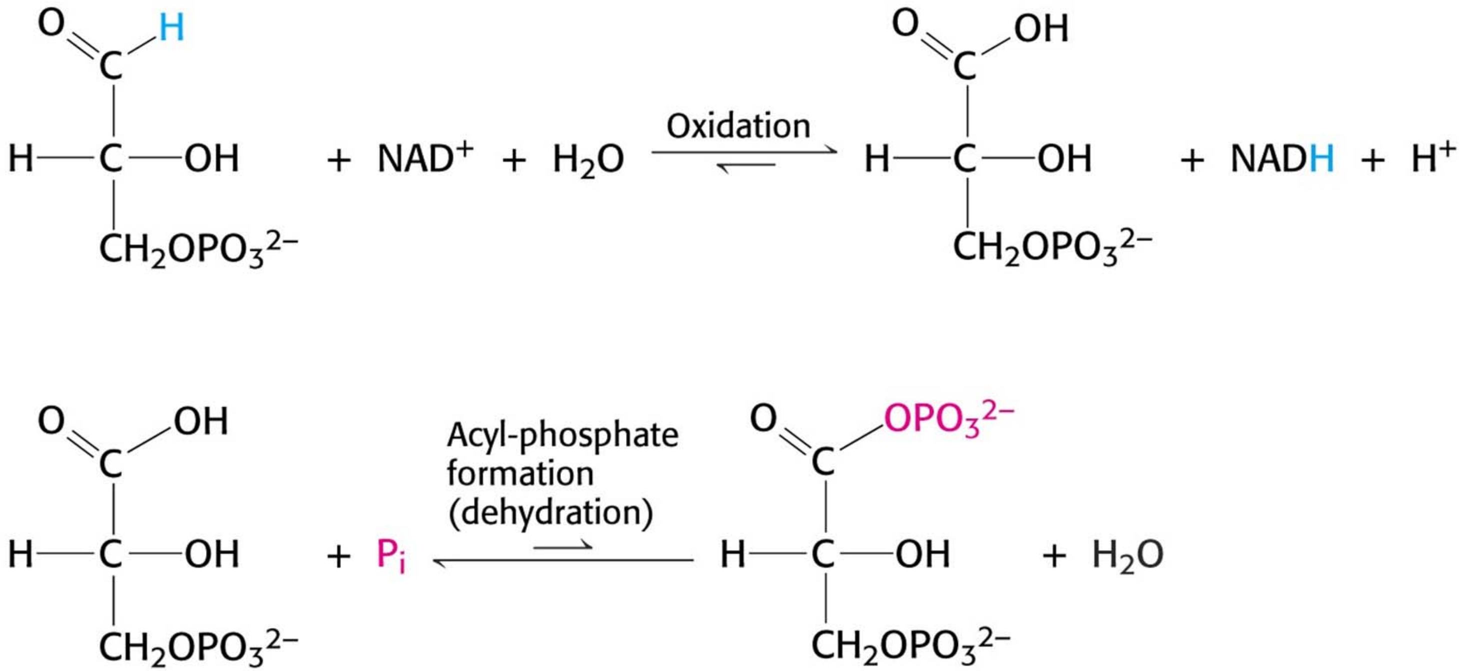
Glyceraldehyde 3-phosphate (GAP)

1,3-Bisphosphoglycerate (1,3-BPG)

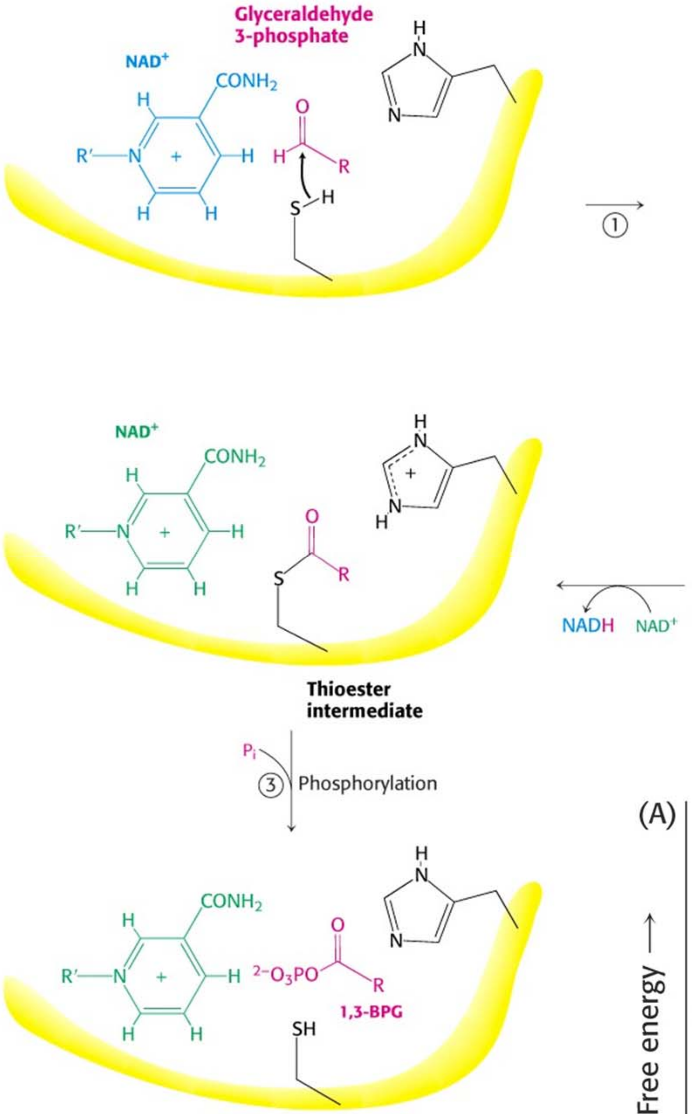
Two-step-reaction:

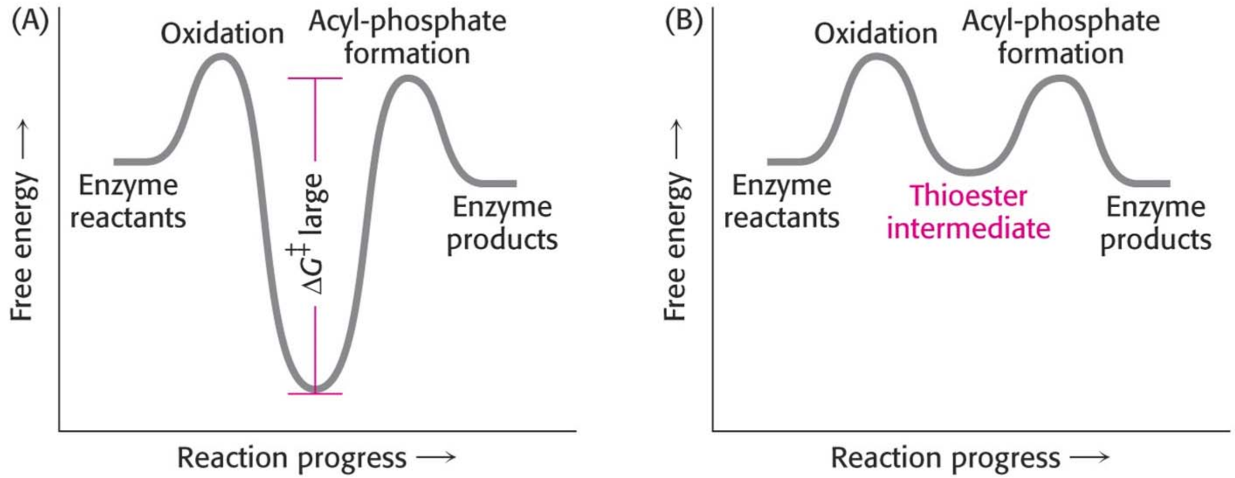
exergonic
$\Delta G^{0\prime} = -43{,}1$ kJ/mol

endergonic
$\Delta G^{0\prime} = +49{,}4$ kJ/mol

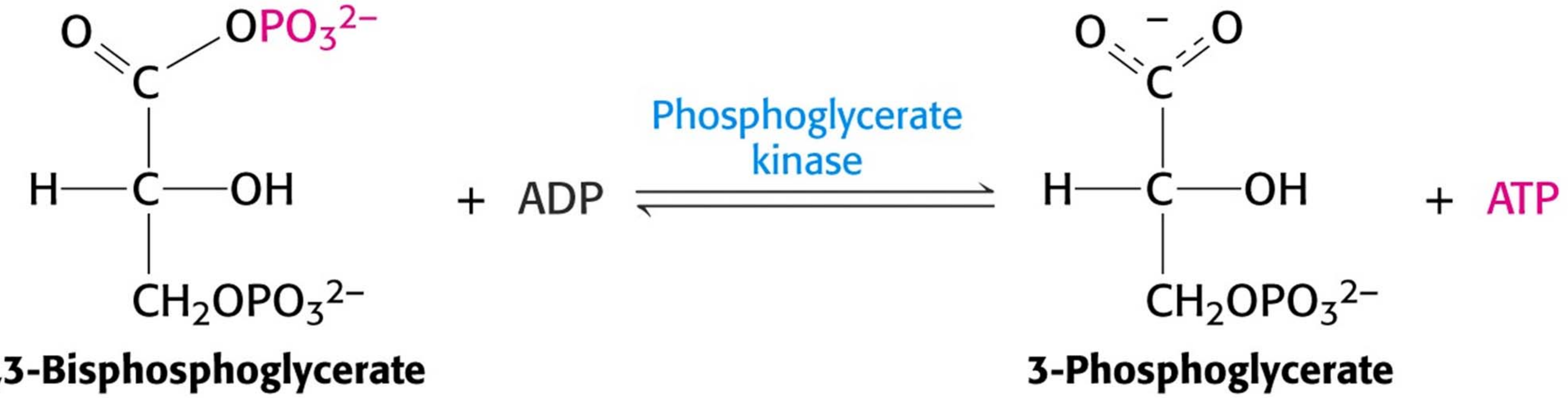


Mechanism of the GAP-DH catalysed reaction

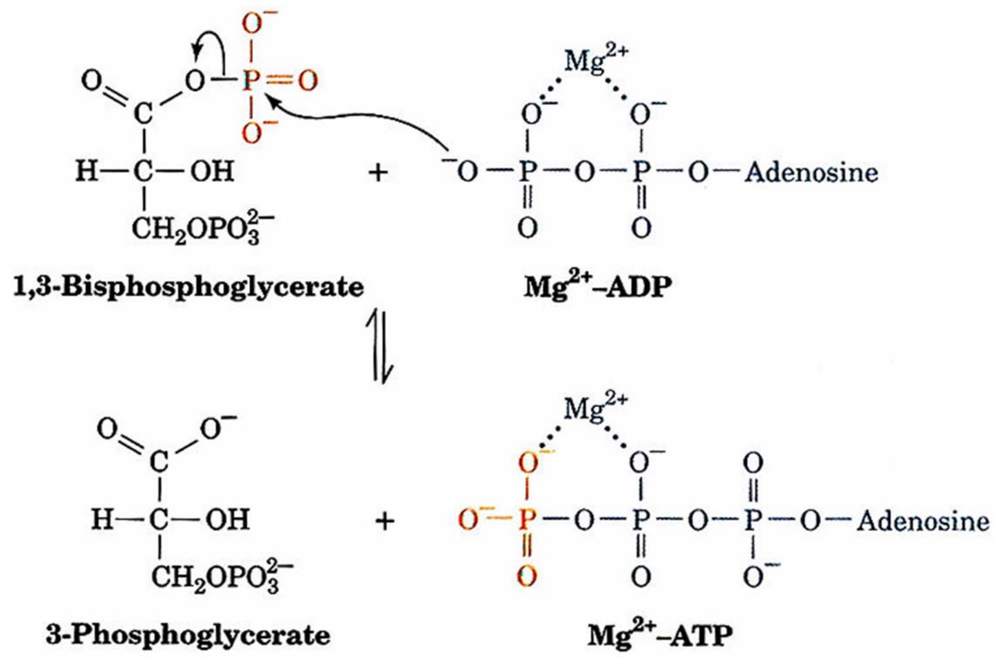




Glycolytic steps: Phosphorylation catalysed by **phosphoglycerate kinase**

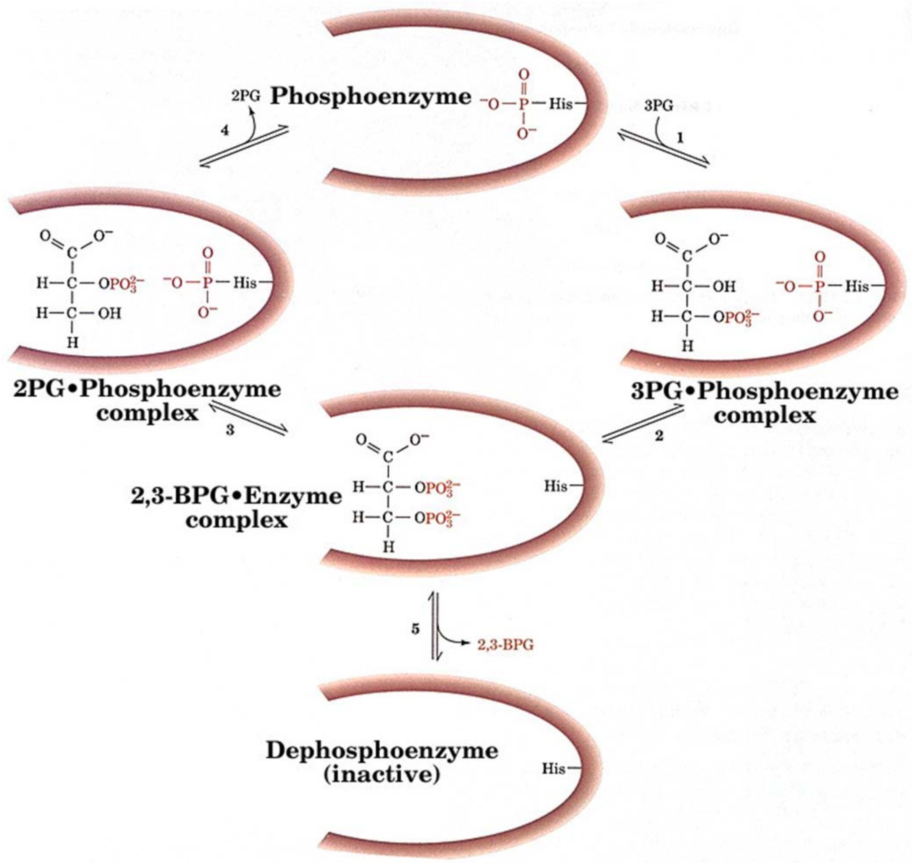


The phosphoglycerate kinase reaction generates the first ATP molecules:

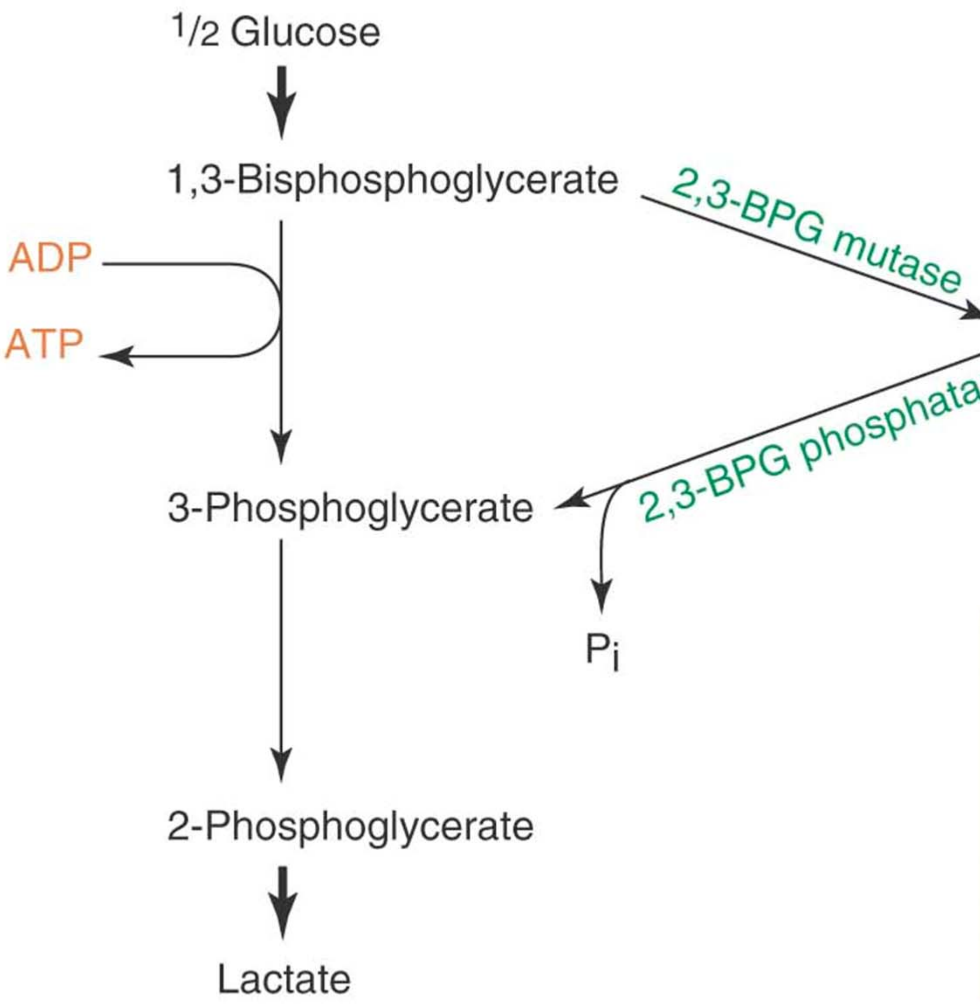


Glycolytic steps: **Phosphoglycerate mutase** catalyzes a phosphoryl group transfer from C3 to C2

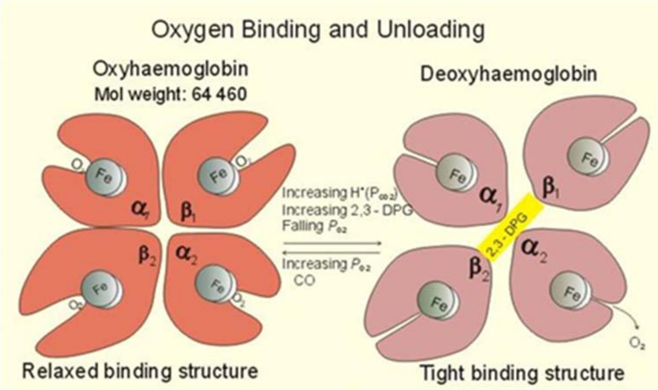
- **Rationale** for this reaction in glycolysis: It repositions the phosphate to make PEP (high energy molecule) in the following reaction (enolase)
- Note the **phospho-histidine intermediates** - a bit of 2,3-BPG (Hb) is required to phosphorylate His (discovered by Zelda Rose)
- ***Nomenclature note****: a "mutase" catalyzes migration of a functional group within a substrate*



The 2,3-BPG Shunt

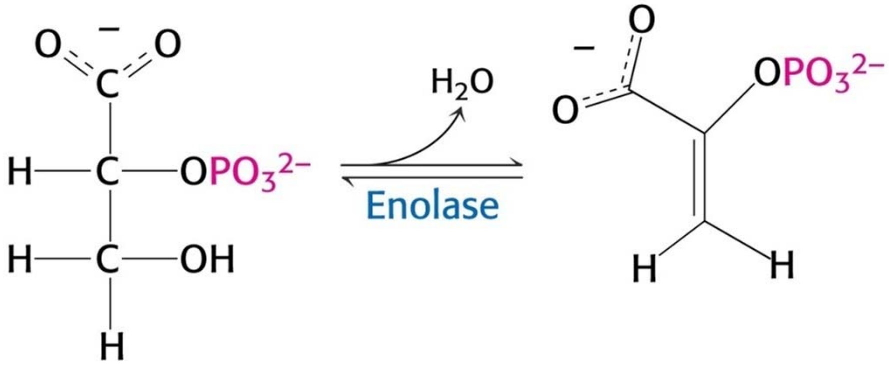


2,3 BPG can prevent tissue hypoxia in conditions of low [O_2].

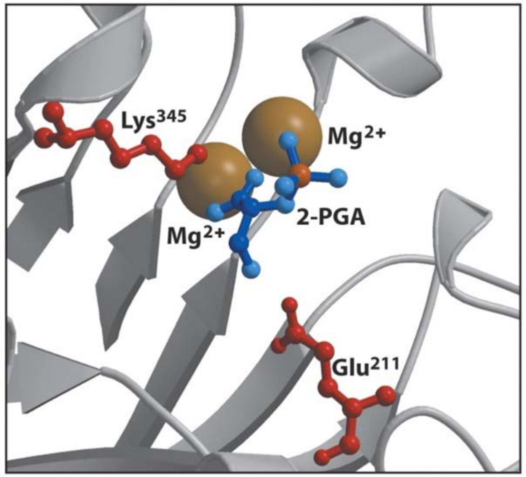


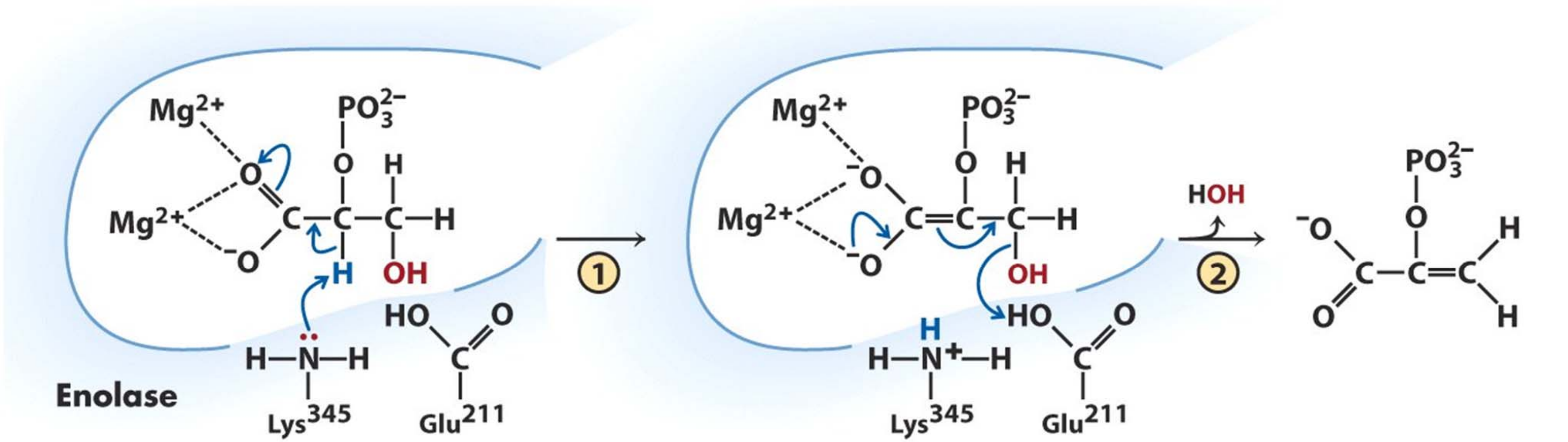
The reactions of 2,3-bisphosphotglycerate (2,3-BPG) shunt are catalyzed by the bifunctional enzyme, 2,3-BPG mutase/phosphatase.

Glycolytic steps: Dehydration catalysed by **enolase**



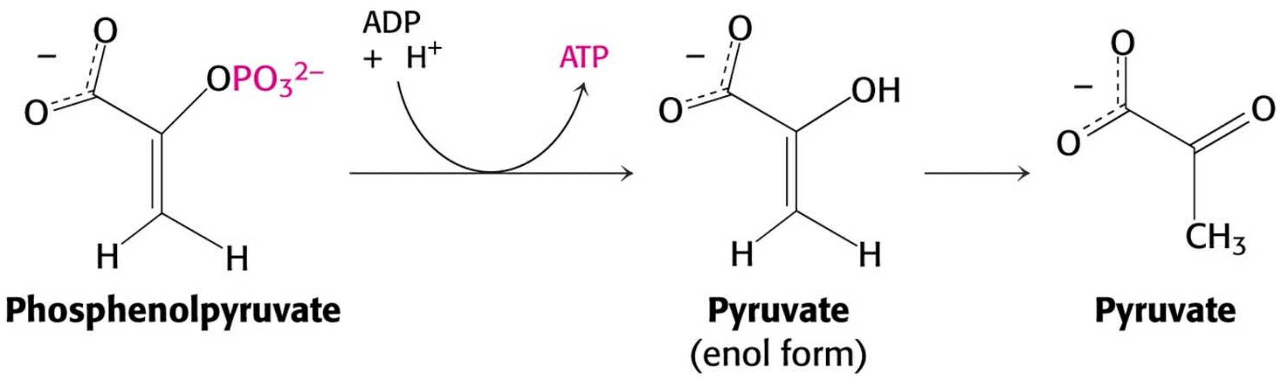
2-Phosphoglycerate **Phosphenolpyruvate**

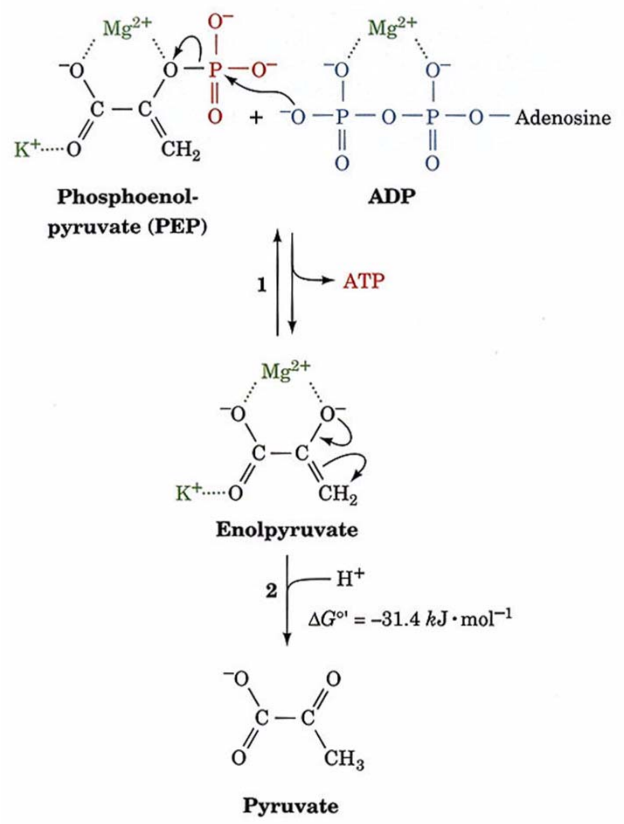




2-Phosphoglycerate bound to enzyme **Enolic intermediate** **Phosphoenolpyruvate**

Glycolytic steps: phosphorylation catalysed by **pyruvate kinase**





The pyruvate kinase reaction converts PEP to pyruvate, driving synthesis of ATP. Pyruvate kinase generates 2 ATP (debt was paid in the phosphoglycerate kinase reaction so both ATPs are a gain of energy).

- enzyme requires Mg^{2+} or Mn^{2+}
- in mammals allosterically regulated:
  - Activators: F-1,6-BP, PEP
  - Inhibitors: ATP, Citrate, Ala